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COMPARATIVE STUDIES ON THE PHYSIOLOGY OF TRICHOMONAD PROTOZOA*

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There has been considerable recent interest in the physiology of trichomonad flagellates (Read and Rothman, 1955; Baernstein, 1955; Ryley, 1955; Wirtschafter, Saltman and Jahn, 1956). However, no attempt will be made in this paper to review the literature on the physiology of these protists. Such a review will be presented elsewhere by Wirtschafter and the author.

The present paper reports some studies carried out with the closely related trichomonad species, *Trichomonas vaginalis* and *T. gallinae,* and a few observations on *T. foetus.* In the work to be reported the first two species were compared with particular respect to carbohydrate metabolism. In axenic culture these organisms require carbohydrate for growth, and it seems probable that carbohydrate is required when the trichomonads are living in a host. Thus, this aspect of metabolism is probably of great importance in the physiology of these animals. *T. vaginalis* and *T. gallinae* have been compared with regard to (1) the utilization of carbohydrate as indicated by the gaseous fermentation of washed, non-proliferating cells; (2) the utilization of various carbohydrates for growth; (3) inhibition of carbohydrate fermentation by various chemical agents; and (4) the presence or absence of the enzymes of the tricarboxylic acid cycle. Some additional data on the carbohydrate physiology of *T. gallinae* will also be presented.

MATERIALS AND METHODS

The *Trichomonas vaginalis* studied was the 5A strain isolated by Wirtschafter and maintained in the C.P.L.M. medium of Johnson and Trussell (1943). The *T. gallinae* used in the experiments was the W.S. strain isolated from a pigeon by Wirtschafter and maintained in the same medium. The latter is a relatively avirulent strain producing mild infections in pigeons and chicks and a slight transient ascites in mice receiving intraperitoneal inoculations of the organism.

In experiments involving the testing of the ability of specific carbohydrates to support growth, *T. vaginalis* and *T. gallinae* were transferred from the C.P.L.M. medium to trypticase medium (Kupferberg *et al,* 1948) from which agar was omitted. In preparation the latter medium was boiled for 1–3 minutes and filtered. Cultures in media without agar were grown in an evacuation jar brought to equilibrium with a 25-pound vacuum. All such cultures had a total volume of 40 ml in 50-ml centrifuge tubes of constricted neck design.

In the initial experiments on the utilization of various carbohydrates for growth the following procedure was used: After 2 48-hour passages in the trypticase medium the cultures were centrifuged at high speed for 10 minutes in the International Centrifuge and the super-

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natant fluid poured off. The cells in each tube were resuspended in approximately 5 ml of sterile Ringer's solution, and pooled in a sterile 125-ml flask. Sterile Ringer's was added to the pooled cells to obtain a concentration of 6.0 to 7.5×10^5 organisms per ml. The test cultures were then prepared in the following manner: 5.0 ml of the cell suspension was pipetted into a sterile 15-ml test tube containing 2.5 ml of human pooled blood serum and 5.0 ml of substrate (equivalent in carbon to 0.44 M hexose). After thorough mixing with a pipette, 2.5 ml of the cell, serum, and substrate mixture were pipetted into each of 4 15-ml culture tubes containing 7.5 ml of sterile stock trypticase medium. The cultures were then incubated under a vacuum of 25 pounds for 48 hours at 37° C. After the incubation each culture tube was processed as follows: The plugs were removed and 1.0 ml of 2% formalin added to each tube with mixing. The tubes were then centrifuged for 8 minutes at high speed in the International Centrifuge. The supernatant was decanted and the lip of the tube wiped with filter paper to remove the last drop. Approximately 2 ml of Ringer's solution was added to each tube and the tube shaken 20 to 30 times, hitting it sharply against the palm of the hand. The suspension was poured into a colorimeter tube calibrated at 5.0 ml. Each culture tube was washed with 2.0 ml of Ringer's solution and the washings added to the colorimeter tube. Ringer's solution was added to each colorimeter tube to the 5.0 ml mark. After thoroughly mixing, each tube was read immediately in the Klett-Summerson colorimeter using the 420 filter. Ringer's solution was used as a blank. A calibration curve for the relation between cell number and photometric readings is shown in Figure 1. The instrument was checked regularly with barium sulphate standards.

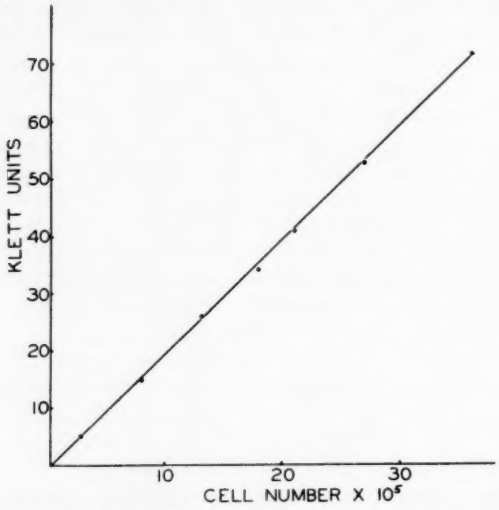

Figure 1. Linearity of photometric determination of cell number. See text for details.

In each lot of experiments the following control tubes were prepared and carried through the entire experiment: (1) maltose as the substrate, (2) Ringer's solution substituted for the substrate, and (3) Ringer's solution substituted for the cell suspension. The last named control always gave a reading which was identical with the blank.

Carbohydrate fermentation was studied using washed 24-hour-old cells from agar-free C.P.L.M. medium. After 3 washings in Krebs-Ringer phosphate by gentle centrifugation the organisms were resuspended in Krebs-Ringer phosphate (pH 6.4) and transferred to Warburg vessels. Substrates were placed in the side arm of the vessels and the vessels gassed with pure nitrogen during 20 minutes' incubation in the 38° C bath. The substrates were added from the side arm and gas evolution followed for a 40-minute period. The total volume of the suspension was 3.0 ml in all experiments. Estimations of carbon dioxide and hydrogen were obtained by using paired flasks, one with and one without 0.2 ml of 30% KOH in the center well. Unless otherwise stated, all substrates were present at a final concentration of 0.02 M. Q values are cubic mm gas per hour per 10^8 organisms.

In manometric experiments involving the use of inhibitors the inhibitor was added from the side arm at the same time that the carbohydrate substrate was added. Glucose was the substrate in all such experiments.

In experiments dealing with the oxidation of di- and tricarboxylic acids, the organisms were harvested from several 24-hour, 40-ml C.P.L.M. cultures, washed with Ringer's solution 3 times by centrifuging and decanting, and resuspended in Krebs-Ringer-phosphate (pH 6.2) to a concentration of 10^7 organisms per ml. One ml of this suspension was added to a tube containing 1.0 ml of Krebs-Ringer phosphate, 0.5 ml of 1% triphenyltetrazolium chloride, and 0.5 ml of 0.1 M substrate (as the sodium salt). The tubes were incubated in the 38° bath for 30 minutes, at which time 1.0 ml of 20% trichloroacetic acid and 2.0 ml of isoamyl alcohol were added to each tube. The tubes were immediately shaken vigorously and allowed to stand for 5 minutes. The alcohol and water layers were separated by centrifugation and the alcohol layer removed to the colorimeter tube. The extraction, with shaking, was twice repeated with 2.0-ml portions of the alcohol and 3 portions added to that of the first extraction. The quantity of extracted formozan was then estimated with the Klett-Summerson colorimeter as suggested by Bodine and Fitzgerald (1949).

In the adaptation experiments with *T. gallinae*, reported in the latter portion of this paper, the cells were counted directly in a haemocytometer chamber. A 1.0-ml aliquot was removed from the thoroughly mixed culture and to it was added 1.0 ml of 2% formalin; quadruplicate samples were counted. These experiments were carried out in quadruplicate.

RESULTS

TABLE I — *Utilization of carbohydrates for growth of* T. gallinae *and* T. vaginalis

Substrate	*T. gallinae*	*T. vaginalis*
Maltose	+29.5±2.1*	+25.2±2.4*
Sucrose	+ 8.0±1.4*	+ 0.5±1.1
Lactose	+ 2.0±1.6	- 3.0±2.1
Trehalose	+10.5±0.8*	+ 9.0±3.8*
Cellobiose	10.4±1.2	—
Melibiose	+ 1.0±0.6	+ 2.5±0.4*
Turanose	+11.0±2.4*	- 1.0±1.2
Glucose	+13.5±1.7*	+ 5.2±1.3*
Galactose	10.7±1.6*	+ 4.5±0.9*
Mannose	+ 1.5±0.8	- 6.0±1.4
Fructose	+13.5±1.1*	- 1.5±1.4
Xylose	- 2.0±1.9	- 1.5±0.8
Arabinose	+ 4.0±1.3*	- 6.5±1.7
Starch	+27.0±2.2	+20.0±0.0*
Glycogen	+20.0±3.1*	+25.1±2.6*
Inositol	0 ±0.0	- 0.5±1.3
d-Sorbitol	0 ±0.0	- 2.5±2.1
Dulcitol	0 ±0.0	- 2.5±1.9

Values are nephelos units of the standard method described in the text. Each value represents the mean of 10 to 12 determinations corrected for control tubes to which Ringer's solution was added instead of substrate. Variation expressed as standard error of the mean.

* - indicates significant utilization.

TABLE II — *Gaseous fermentations of trichomonads*

Substrate	*T. gallinae*		*T. vaginalis*	
	Q_{H_2}	Q_{CO_2}	Q_{H_2}	Q_{CO_2}
Glucose	100*	100	100*	100
Galactose	121*	105	19	20
Mannose	19	18	20	18
Fructose	118*	110	18	17
Arabinose	20	25	10	16
Xylose	50*	61	18	19
Maltose	102*	98	104*	96
Trehalose	98*	100	50*	60
Lactose	15	17	18	21
Sucrose	64*	66	22	17
Starch	102*	97	—	—
Glycogen	90*	88	75*	60
Endogenous	18	16	20	18

Mean values of 4 to 6 determinations with each substrate, based on relative rate with glucose as 100.

* = significant fermentation above endogenous values.

Utilization of Carbohydrates for Growth

The utilization of various carbohydrates as energy sources for the growth of *T. vaginalis* and *T. gallinae* as indicated by the number of cells in a 48-hour culture is shown in Table I. It may be seen that the most efficient substrates for *T. vaginalis* are maltose and glycogen. Starch consistently gave slightly less growth. Still less growth was obtained with trehalose, glucose, galactose, and melibiose. None of the other carbohydrates or the sugar alcohols were utilized by *T. vaginalis*. *T. gallinae* is somewhat less exacting in its carbohydrate requirements. Maltose, starch, and glycogen were the most efficient substrates. Glucose, galactose, fruc-

tose, turanose, sucrose, cellobiose, and trehalose were utilized to a lesser extent. Arabinose was used to a small but significant extent. None of the other carbohydrates or sugar alcohols tested were used by *T. gallinae*.

The effects of varying the maltose concentration on growth of *T. gallinae* are shown in Figure 2. To determine whether concentration effects may be involved in the differences observed in growth when glucose, maltose, or sucrose are added to the medium, growth at a number of sugar concentrations, above that indicated to be limiting in the case of maltose, was determined. These data are presented in Figure 3. In each case the osmotic concentration was maintained by the addition of sorbitol so that the total osmotic concentration of added carbohydrate material was initially equal to 0.088 M. It is obvious that increasing the carbohydrate concentration in this range is without effect. Further, the pH of cultures of *T.*

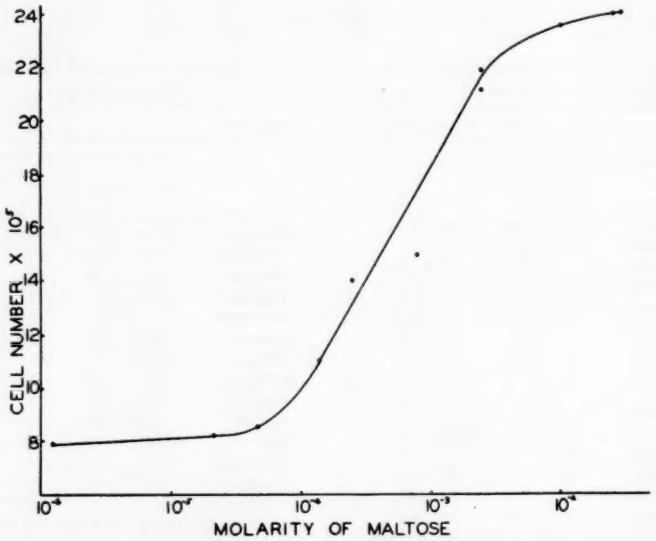

Figure 2. The effect of various concentrations of maltose on the number of cells in a 48-hour-old culture of *T. gallinae*. Cell number determined by count; inoculum, 7.8×10^{-5} cells thrice washed in Ringer's solution. Each point is average of quadruplicate determinations.

gallinae growing in the presence of maltose and glucose underwent very small changes during the cycle of growth: Measurements at 6-hour intervals during growth were made on a number of such cultures. The growth of neither *T. vaginalis* nor *T. gallinae* was affected by the addition of lactose to media containing an equal concentration of maltose nor by the addition of the glucosides, phloridzin or salicin, at concentrations up to 0.03 M. Addition of alpha-methylglucoside at a concentration of 0.03 M inhibited the growth of *T. gallinae* 30 to 50%. A detailed analysis of the inhibition by the determination of generation time at several concentrations of the inhibitor in the presence of varying quantities of maltose was attempted. The results were not entirely satisfactory from the standpoint of interpretation. An analysis of the data suggests that the inhibition is of a type which has been termed uncompetitive (Massart, 1950).

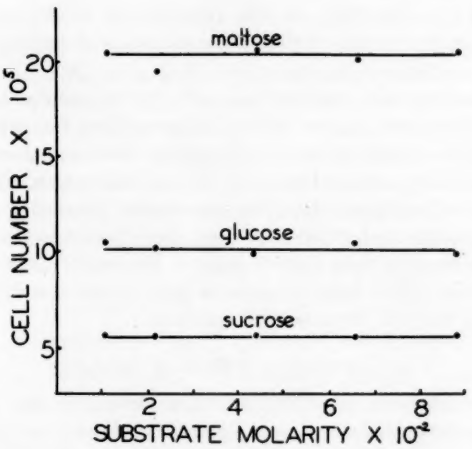

Figure 3. The effects of varying concentrations of maltose, glucose, and sucrose in the non-limiting range on the number of cells per ml in 48-hour old cultures of *T. gallinae*. Each point is average of quadruplicate determinations.

Utilization of Carbohydrate in Gaseous Fermentation

Although it has been determined in the present studies that, under anaerobic conditions, *T. gallinae* produces a gas that is absorbed in neither alkali nor acid, it has not been shown by appropriate analytical methods that the gas is hydrogen. However, since *T. vaginalis*, and *T. foetus* produce hydrogen (Suzuoki and Suzuoki, 1951; Read and Rothman, 1955), the gas which is produced by *T. gallinae* and which is not absorbed by alkali, is referred to as such in this paper. Cells of *T. gallinae* carry out a vigorous gaseous fermentation of glucose. Cells harvested after 24 hours' growth in C.P.L.M. medium and washed by repeated centrifugation have a $Q_{CO_2}^{N_2}$ of 644 and a $Q_{H_2}^{N_2}$ of 485 (average of 9 determinations in each case) in the presence of 0.01 M glucose at pH 6.0. In the absence of glucose such cells

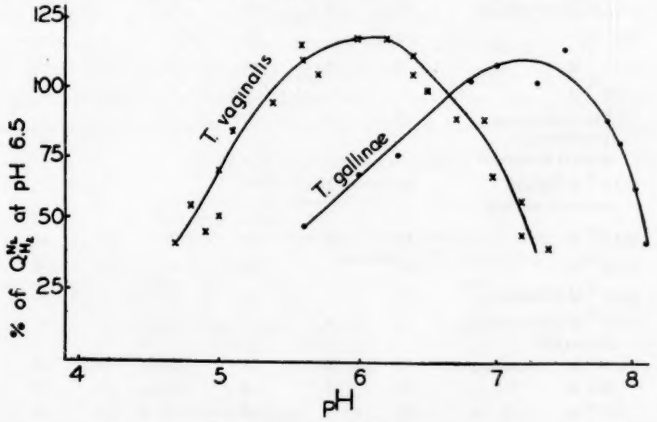

Figure 4. The effect of pH on the evolution of hydrogen by *T. vaginalis* and *T. gallinae* in the presence of 0.02 M glucose.

have a $Q_{CO_2}^{N_2}$ of 235 and a $Q_{H_2}^{N_2}$ of 175 (average of 10 determinations in each case). The comparative effects of pH on the evolution of hydrogen by *T. gallinae* and *T. vaginalis* are shown in Figure 4.

In short experiments with non-dividing cells, *T. vaginalis* and *T. gallinae* were found to ferment the same sugars which can be utilized for growth (Table II). From the qualitative standpoint the two different determinations yield the same information as to which carbohydrates are utilized and which are not. However, from the quantitative standpoint there are some rather interesting differences. The fermentability of glucose and maltose in short experiments is not significantly different. However, in supporting growth maltose is a much more efficient substrate than glucose, yielding about 5 times as many cells in the case of *T. vaginalis* and more than twice as many in the case of *T. gallinae*.

The Comparative Effects of Inhibitors

A number of compounds have been tested to determine their ability to inhibit the gaseous fermentations of *T. vaginalis* and *T. gallinae*. In reporting the data, a few observations on *T. foetus* (G) have also been included. (Table III) It

TABLE III — *Effects of inhibitors on gaseous glucose fermentation of tricbomonads*

Inhibitor	%Inhibition					
	T. gallinae (WS)		*T. vaginalis* (5A)		*T. foetus* (G)	
	Q_{H_2}	Q_{CO_2}	Q_{H_2}	Q_{CO_2}	Q_{H_2}	Q_{CO_2}
10^{-3} M Arsenate	0	0	0	0	-	-
10^{-3} M Arsenite	0	0	48	10	-	-
$5x10^{-3}$ M Fluoride	85	98	51	77	0	0
10^{-3} M "	22	4	13	0	0	0
10^{-4} M Iodoacetate	100	98	100	100	-	-
10^{-5} M "	-	-	47	+31	-	-
10^{-2} M Glyceraldehyde	0	0	0	0	-	-
10^{-3} M Dinitrophenol	58	24	100	21	-	-
10^{-4} M "	17	7	52	+35	-	-
$5x10^{-5}$ M "	0	0	46	0	-	-
10^{-5} M "	-	-	0	0	-	-
10^{-4} M Carbarsone (p-carbamino-phenyl arsonate)	0	0	0	0	-	-
$5x10^{-3}$ M Phenyl mercuric acetate	98	37	-	-	-	-
$5x10^{-4}$ M "	39	27	100	100	67	42
$5x10^{-5}$ M "	-	-	70	0	21	0
$3x10^{-2}$ M Phloridzin	0	0	0	0	-	-
$3x10^{-2}$ M Alpha-methyl glucoside	0	0	0	0	-	-
$2x10^{-2}$ M Cinnamate	77	70	25	10	40	31
10^{-2} M "	55	51	0	0	32	29
10^{-3} M "	25	5	0	0	10	10

Mean values of 3 to 4 determinations in each case. Inhibition expressed as percent of control containing glucose as substrate without inhibitor. Values with + indicate stimulation. See text for details of methods used.

may be seen that there are some definite differences in the susceptibility to various compounds. *T. gallinae* is unaffected by arsenite (10^{-3} M) while hydrogen evolution of *T. vaginalis* is rather sharply affected. Gas evolution of *T. gallinae* is markedly inhibited by fluoride at a concentration of 5×10^{-3} M, that of *T. vaginalis* is somewhat less affected, and gas evolution of *T. foetus* is not affected at all. It is of interest that metabolism of *T. gallinae* and *T. vaginalis* is not inhibited by glyceraldehyde at 10^{-2} M concentrations; this compound inhibits glycolysis in many other organisms. *T. vaginalis* is somewhat more sensitive to 2,4-dinitrophenol and phenylmercuric acetate than *T. gallinae*. *T. foetus* seems to be intermediate in its sensitivity to the latter compound. On the other hand, *T. gallinae* is more sensitive to cinnamate than either *T. vaginalis* or *T. foetus*.

Adaptation to Poorly Utilized Carbohydrates

The experiments on the growth of these trichomonad species with different carbohydrates as energy sources were carried out with maltose-grown cells. It seemed desirable to determine whether *T. gallinae* would show enhanced growth after being grown for several transfers in media containing sugars previously shown to support submaximal growth. The data obtained may be summarized as follows: Upon the second transfer in media containing galactose *T. gallinae* grows at an increased rate and attains populations which are not significantly different from those obtained in maltose-containing media. Repeated transfers of *T. gallinae* in media containing glucose have no effect on the growth rate nor total cells produced in subsequent transfers. In the same fashion, after 48 transfers in media containing sucrose *T. gallinae* showed neither an increase in growth rate nor in final number of cells produced, above what was observed in the initial transfer to sucrose from maltose-containing media.

The Tricarboxylic Acid Cycle

Wirtschafter, Saltman, and Jahn (1956) have recently reported on the failure to demonstrate the tricarboxylic acid cycle in *T. vaginalis* (5A). These workers used C-14-labeled substrates in the studies which are regarded by the writer as convincing. In the present work their findings were corroborated by a different procedure, and it was readily demonstrated that *T. gallinae* (WS) could oxidize a number of the intermediates of the tricarboxylic acid cycle. The method used in estimating the reduction of triphenyltetrazolium has been described above. Typical results are shown in Table IV. No special significance can be attached to the

TABLE IV — *Oxidation of organic acids by trichomonads*

Substrate added	*T. gallinae*	*T. vaginalis*
Citric	227	100
Isocitric	182	100
Alpha-ketoglutaric	191	100
Succinic	218	100
Fumaric	145	100
Malic	295	98
Beta-hydroxybutyric	195	99
None	100	100

Relative reduction of triphenyltetrazolium chloride in the presence of substrates. See text for details.

differences in the quantity of formozan produced by *T. gallinae* in the presence of various substrates. This is admittedly an artificial system and, since whole cells were used, these differences could be due to differences in penetration of the substrates rather than to any real difference in intracellular enzyme activity. The data indicate that there is a marked difference between *T. gallinae* and *T. vaginalis* in the complement of respiratory enzymes. These observations of no activity, in the case of *T. vaginalis,* might also be attributed to lack of permeation of the substrates. However, Wirtschafter, *et al* incubated *T. vaginalis* (5A) with pyruvate labeled with C-14, pyruvate being readily metabolized by the organism, and ascertained the fate of the label by chromatography. Tricarboxylic acid cycle intermediates were neither present nor labeled in incubated cells, although these compounds were readily demonstrated after addition to the mixture. In view of the present observations on *T. gallinae* and *T. vaginalis* and those of Wirtschafter *et al,* on the latter species, it is concluded that these two species differ in that *T. gallinae* has the enzymes of some form of the tricarboxylic acid cycle while *T. vaginalis* lacks these enzymes, with the possible exception of malic dehydrogenase. It is of interest to note that fluoroacetate, a potent inhibitor of the tricarboxylic acid cycle in many organisms, has no effect on the aerobic respiration of *T. gallinae* at concentrations up to 10^{-2} M.

During the present study several attempts were made to demonstrate the oxidation of di- and tricarboxylic acids by *T. foetus,* using the tetrazolium technique. However, the endogenous rate of reduction was extremely high and the results were equivocal. Ryley (1955) reported similar experience, using methylene blue and ferricyanide reduction as criteria of dehydrogenase activity in *T. foetus.*

DISCUSSION

T. vaginalis and *T. gallinae* show a number of interesting differences in the gross aspects of their physiology. The report of Trussell and Johnson (1941) that *T. vaginalis* will utilize glucose and its polymers has been corroborated in the present study. The organism is not, however, completely restricted in this respect. *T. vaginalis* will also utilize galactose and melibiose (D-glucose-6-αD-galactopyranoside) to a small but significant extent. On the other hand, *T. gallinae* is capable of utilizing several monosaccharides and disaccharides not utilized by *T. vaginalis.* Our results with *T. gallinae* show general qualitative agreement with those of Cailleau (1935), with certain minor exceptions. Since Cailleau's data were semi-quantitative, detailed comparison does not seem profitable. Comparison of the results obtained in determining the utilization of sugars for growth and for short term fermentation by washed non-proliferating cells reveals that these two experimental methods yield the same qualitative information but somewhat different quantitative results. Thus, though *T. gallinae* and *T. vaginalis* ferment glucose and maltose at essentially the same rate in experiments of short duration, during growth maltose produces twice the population, in the case of *T. gallinae,* and 5 times the population, in the case of *T. vaginalis,* obtained when glucose is the carbohydrate added to the medium. It should also be pointed out that starch and glycogen yield populations of the same magnitude as maltose while trehalose and cellobiose resemble glucose in their efficiency as carbohydrate sources for growth. Starch, glycogen, and maltose have in common a 4,1 β glucoside linkage between the glucose units. Trehalose has a 1,1 linkage between the glucose units. Cello-

biose resembles maltose in having a 4,1 linkage between the glucose units but has an α glucoside configuration. Thus, the data support the idea that the 4,1 β glucoside linkage between glucose units is more utilizable in terms of growth than are other linkages. More than this, the fact that greater growth is obtained with carbohydrates containing the glucose 4,1 β glucoside configuration than with glucose suggests that the configuration of this carbon linkage is of some positive chemical value other than the mere possession of a highly efficient system for hydrolysis (e.g. β-glucosidase). These organisms have transglycosidases which may be involved. Data on the transglycosidases will be published elsewhere. The experiments dealing with the effects of carbohydrate concentration further indicate that the concentration probably plays no role in the differential growth-supporting effects of glucose 4,1 β glucosides.

Though there are quantitative differences in the effects on gaseous fermentation of the inhibitors tested, the trichomonads show considerable qualitative similarities. An exception to this is seen in the effect of fluoride which inhibits the fermentations of *T. gallinae* and *T. vaginalis* but has no apparent effect on that of *T. foetus*. Ryley (1955) reported that fluoride did not affect the anaerobic metabolism of *T. foetus*.

The evidence of some form of the tricarboxylic acid cycle in *T. gallinae* is in some contrast with the report of Willems, Massart, and Peeters (1942) that *T. hepatica* (= *T. gallinae*) does not possess these enzymes. These authors based this conclusion on the finding that the oxygen consumption of the trichomonad was not increased by the addition of pyruvate or succinate: The average Q_{O_2} reported by Willems and his coworkers was 600 which is essentially identical with values we have obtained with *T. gallinae* in the presence of glucose. It seems probable that the cells studied by Willems *et al* were respiring at the maximum rate before the addition of succinate or pyruvate.

The most striking difference between *T. vaginalis* and *T. gallinae* is the apparent absence of the tricarboxylic acid cycle in the case of the former species and the presence of these enzymes in the latter. This seems to parallel the situation reported for various groups of species of trypanosomes. More information on other trichomonads will be required before it can be determined that such a criterion would serve a specific purpose in the taxonomy of this protozoan group. It seems plain that biochemical comparisons of protozoans will eventually be useful in taxonomy in much the same way that such characteristics have proven of primary use to the bacteriologist. The protozoologist has, in addition, the advantage of at least a few more morphological characters than the students of the bacteria.

SUMMARY

The utilization of various carbohydrates as energy sources for growth of *Trichomonas vaginalis* and *T. gallinae* has been quantitatively compared. *T. vaginalis* utilizes glucose and its polymers, galactose, and melibiose. A variety of other sugars and sugar alcohols are not utilized. *T. gallinae* utilizes glucose and its polymers, galactose, fructose, sucrose, turanose, and arabinose. Some other carbohydrates and the sugar alcohols are not utilized by *T. gallinae*. Glucose polymers having the glucose-4,1-β-glucoside configuration are most efficient in stimulating growth. It was shown that this is not related to effective concentration of substrate nor to pH alterations of the medium.

Using gaseous fermentation as a measure of carbohydrate utilization, the sugars utilized are those which support growth. However, the relative quantitative differences in rates of utilization as determined by short fermentation experiments do not correspond to those obtained in growth experiments.

The gaseous fermentations of *T. vaginalis* and *T. gallinae* are inhibited by fluoride, iodoacetate, dinitrophenol, and phenymercuric acetate. They are not inhibited by arsenate, arsenite, glyceraldehyde, carbarsone, phloridzin, or alpha-methylglucoside. There are quantitative differences in sensitivity. *T. foetus* is not inhibited by fluoride at relatively high concentrations.

Attempts to adapt *T. gallinae* to poorly utilized carbohydrates showed that after a single transfer the organism showed an enhanced capacity for growth on galactose. However, after 48 transfers in sucrose media no enhancement of growth on this substrate was observed.

Using a tetrazolium assay, oxidation of Krebs cycle intermediates was demonstrated in *T. gallinae* but could not be demonstrated in *T. vaginalis*.

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HOST RESISTANCE AND REGULATION OF THE DEVELOPMENT OF *PLASMODIUM LOPHURAE* IN PHEASANTS, COOTS AND DOMESTIC PIGEONS*

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To know the nature of a parasite one must study it under all conditions compatible with its development. Plasmodia are related to their hosts in a highly specialized manner, and their mode of reproduction enables one to study quantitatively certain fundamental aspects of their behavior in all possible host-parasite combinations. This problem of biological inter-relations was undertaken to determine the effects of various hosts upon the course of development of *P. lophurae* as reflected by: (1) the rate of reproduction; (2) the course and intensity of parasitemia; (3) the destruction and survival of parasites; and (4) the percentage frequency of merozoite groups.

Plasmodium lophurae (Coggeshall, 1938) is capable of reproducing in diverse avian hosts. Indeed, its hosts have lately been extended to include mice (McGhee, 1951). This indicates an unusual adaptability of the schizogonous cycle; therefore it is not surprising that it displays little avian host specificity. Considerable work has been done on various aspects of the biology and morphology of *P. lophurae* but detailed studies of the asexual cycle have been carried out using ducks and chickens as hosts.

The development of *P. lophurae* was studied in 15 species of birds: Pigeons, pheasants, coots, chickens, house finches, canaries, white- and golden-crowned sparrows, California quail, brown towhees, horned owls, fish crows, Oregon juncos, Eastern jays and English sparrows. With the exception of reference to some merozoite means (Table I) data from only the first 4 are included in this paper, and discussed with respect to host resistance.

MATERIALS AND METHODS

The 12A strain of *P. lophurae,* isolated from the pheasant *Lophura igniti igniti* by Coggeshall in 1938, was used in these studies. All infections were blood-induced and studied from blood films made at 4-hour intervals. Either 2 blood films were made at each interval and 1 hemolyzed, or 1 slide was made at each interval and 1 end of it hemolyzed to facilitate merozoite-counting as described by G. H. Boyd (1939). The area on the single smear at the junction of the etherized and non-etherized portion is especially useful, since the fumes from the ether gently erupt or merely distend the red blood cells, disposing the merozoites in the same plane. This practice not only facilitates counting but it also makes for accuracy, since it precludes the possibility of loss of merozoites from mature segmenting groups. Slides were stained with either MacNeal's tetrachrome or with Giemsa after fixation with methyl alcohol, and studied with the oil immersion objective and 8× oculars.

Since malarial parasites reproduce by multiple division in the blood stream, reproductive events and loss of parasites may be followed throughout the course of

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development from blood films made at regular intervals. Inasmuch as counts were calculated per 10,000 red blood cells without reference to red blood cell counts, this method is open to criticism as is the practice of using wild birds for an experimental study. However, in a comparative study of this sort it was considered sufficient to make the counts from prepared slides without considering the variable produced by anemia. The techniques employed in handling the parasite excluded the probability of complications brought about by naturally infected wild birds. All wild birds were kept in captivity for several weeks and in some cases for several months before use, and observations on their blood were made at regular intervals previous to infecting them with *P. lophurae*. Infections were established in the experimental birds by intravenous injections of enough infected blood so that parasites could be found immediately after injection and followed in their development.

Although only uninucleate forms and mature segmenters were used in determining cycles, and in calculating survival and death rates, parasites were divided into the following growth-stage groups for study in order to establish a perspective of reproduction in the various hosts: (1) uninucleate forms; (2) schizonts containing 2–6 nuclei; (3) multinucleate schizonts; and (4) mature segmenters. To obtain the length of the asexual cycle the peaks of mature segmenters were used. Obviously the peaks of multinucleate schizonts could not be used for this, since the number of nuclei of a parasite reproducing at the rate of 11 merozoites per segmenter every 56 hours (as for example, *P. lophurae* in pigeons) would not be comparable in stage of development to one with the same number of nuclei if the latter were reproducing at the rate of 15 merozoites per segmenter every 32 hours (as for example, *P. lophurae* in coots). Because there are marked variations in the rate of reproduction of the same parasite in various hosts, the only comparable stages in the course of development in the hosts studied are the mature segmenters and merozoites. It was from the peaks of uninucleate parasites represented in the graphs as "rings" per 100 parasites that the survival and destruction rates were obtained. The population of uninucleate parasites represents the number facing the problem of survival in the host during the time it takes them to reach maturity and reproduce. If they all live to segment, parasitemia should increase at the end of each cycle by a number equivalent to the average number of merozoites being produced by each segmenter at that time. Counts were made of mature segmenters per 10,000 parasites and expressed in the graphs as segmenters per 1,000 parasites.

Merozoite means were obtained from counts of 50 random mature segmenters from hemolyzed blood films throughout the infection. Means of a few merozoite groups containing only 25 or 36 segmenters were obtained when segmentation was lowest, but not many. Usually more than 200 segmenters were counted every day for every infection as long as it lasted. Counts were made only from completely separated merozoites, and standard errors were calculated.

Parasitemia was determined by finding the ratio of parasites to red blood cells. Counts were made on parasites per 10,000 red blood cells and expressed in the graphs as parasites per 1,000 red blood cells. Generally, counts were kept within a 10% error; it never exceeded an error of 15% according to the method of counting developed by Gingrich (1932). The increase in parasitemia was obtained by actual count at the beginning and at the end of each cycle. The rate of increase representing the survival of parasites for that segmentation was then subtracted

from the merozoite mean to determine those which were lost. The percentage of parasites destroyed during that time may be found by dividing the number lost by the number produced.

Blood was obtained by cardiac puncture. An anticoagulant of a 1:1 mixture of 2% sodium citrate and normal saline was used. All red blood cells were washed once in this mixture by centrifugation. After the supernatant fluid was removed, a volume of the citrate-saline mixture was added to the red blood cells in amounts about equal to that of the volume of cells, and injected immediately by the intravenous route.

EXPERIMENTAL RESULTS

TABLE I—*Average number of merozoites formed by each segmenter of* Plasmodium lophurae *developing in different avian hosts*

	Plasmodium lophurae		
Hosts	**Number of segmenters examined**	**Mean**	**Range**
Coots	3,233	15.43 ± .11	5–36
Pheasants	4,147	13.77 ± .06	6–36
Chickens	5,080	11.71 ± .02	6–21
Pigeons	5,287	11.05 ± .05	4–18
House finches	2,000	11.50 ± .05	6–20
White-crowned sparrows	560	12.16 ± .03	6–18
Golden-crowned sparrows	500	9.28 ± .07	5–14
California quail	523	12.74 ± .08	8–24

I. *Plasmodium lophurae* in chickens

The somewhat asynchronously reproducing plasmodium produces an infection characterized by (1) an acute rise (2) a peak in numbers (3) a parasite decline, and (4) a latent infection. The length of the asexual cycle was found to be about 36 hours as previously reported by Hewitt (1942) in ducks, and by Taliaferro and Taliaferro (1940, 1950) in chickens.

Figure 1 represents composite data from 6 chickens weighing 220 to 250 grams and kept under 12 hours of light and 12 hours of darkness. Under these conditions the mean number of merozoites produced by each schizont was 11.71 ± .02, ranging from 6 to 21 (Fig. 7). Segmenters with more than 14 merozoites were uncommon. Thirty-five percent of more than 5,000 merozoite groups counted contained 12 merozoites. The length of the asexual cycle during the rise of parasitemia was from 36 to 40 hours, but there was a delay in frequency of schizogony during and following the crisis which amounted to from 21% to 32% decrease in the rate of reproduction. In addition to the decrease due to delays in schizogony, there was an abrupt drop in the number of merozoites produced by each segmenter at the crisis as represented by the broken line, and an increase in the destruction of parasites as represented by the lined area in Figure 1. In this host the parasiticidal antibodies of acquired immunity are not strong enough to sweep the parasites from the blood stream immediately; therefore they appear in the blood as injured parasites which grow more slowly than usual and ultimately produce segmenters with fewer merozoites (Taliaferro and Taliaferro, 1950). The smallest merozoite mean produced by this inhibition of reproduction was 9.50, while during the initial infection each segmenting schizont produced on the average 12.35 merozites. This inhibition of reproduction was responsible for about a 15% decrease. During the patent period there was on the average a 3- or 4-fold increase in parasites following seg-

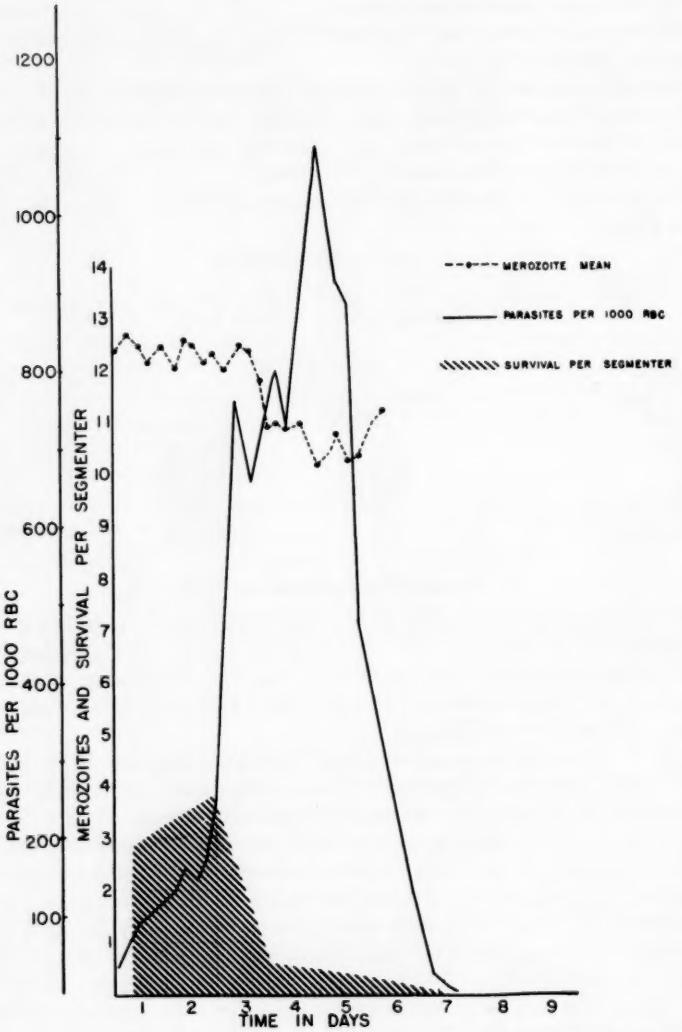

FIGURE 1. Course of development, parasitemia, merozoite mean, survival and death per segmenter of *P. lophurae* developing in chickens.

mentation, and never the 12-fold increase which would have resulted if none of the parasites had been destroyed. Hence about 8 out of the 12 produced were destroyed during the acute rise of the infection before acquired immunity was established.

During the crisis large numbers of mitotic medium-sized and small lymphocytes may be seen in the blood as well as many extremely pyknotic and contracted parasites referred to as crisis forms by Taliaferro and Taliaferro (1934). The blood cells whether parasitized or not at this time are variously distorted and often fragmented.

Data concerning the rates of reproduction, survival and destruction of *P. lophurae* in chickens corroborate those previously published by Taliaferro and Taliaferro (1950) and are included here to form a baseline comparison for data obtained and analyzed similarly from other avian hosts in which the course of *P. lophurae* has not, to the author's knowledge, been previously studied.

II. *Plasmodium lophurae* in Chinese pheasants (*Phasianus colchicus*)

The course of infection was studied in 15 pheasants 3 to 4 weeks old. Under the conditions of a 12-hour night and day schedule, the peaks of mature segmenters are from 36 to 48 hours. In 3 pheasants 2 broods of reproducing parasites were noted. Figure 2, incorporating data obtained from 6 pheasants 4 weeks old, shows that in general the survival rate of parasites is low, while the reproduction rate is uniformly high. During the first and second segmentations in this group respectively 3.10 and 2.80 merozoites per segmenter survived; during the third the survival rate fell to less than 1. Table II summarizes survival per segmenter for successive cycles for all pheasants studied. The merozoite mean of more than 4,000

TABLE II—*Reproductive capability of* Plasmodium lophurae *developing in three different avian hosts and survival per segmenter for successive cycles*

	Birds infected with *Plasmodium lophurae*					
Cycles	Coots 15.43 ± .11*		Pheasants 13.77 ± .06*		Pigeons 11.05 ± .05*	
	Number living	Number lost	Number living	Number lost	Number living	Number lost
1	11.88	3.55	3.70	10.07	5.64	5.36
2	3.85	11.58	3.40	10.37	2.66	8.34
3	.85	14.58	1.00	12.77	1.84	9.16
4			.30	13.47	.50	10.50
5					.35	10.65
6					.01	11.04
	Length of cycle 32–36 hours		Length of cycle 36–40 hours		Length of cycle 48–56 hours	
	Peak of parasitemia 10,113		Peak of parasitemia 3,457		Peak of parasitemia 5,928	

* merozoite mean

merozoite groups examined from pheasants throughout the infections was 13.77 ± .06, ranging from 6 to 36 (Fig. 6 and Table II). Of these merozoite groups about 35% contained 14 merozoites, and 25% contained more than 14. In general, pheasants destroy from 75% to 80% of the merozoites produced during the acute rise.

Pheasants fully recovered from infections with *P. lophurae* were superinfected so that several parasites per microscopic field could be found immediately after superinfection. The merozoites produced by the progeny of this first segmentation were counted and found to be about the same as the merozoite mean obtained from the initial infections in the same birds. For example, the average number of merozoites per schizont in all superinfected pheasants was 13.60 ± .09 as compared to 13.77 ± .06 in initial infections. Similarly the merozoite mean of initial and superimposed infections was compared in individual pheasants. Pheasant number 54 was superinfected so that 1,700 parasites per 10,000 red blood cells could be counted a few hours following injection of parasites. These parasites lived just long enough for merozoite means of only 1 segmentation to be counted. The merozoite mean per segmenter in this pheasant during the initial infection was 13.00, while that of the superimposed infection was 13.30. The strong parasiticidal effects of innate im-

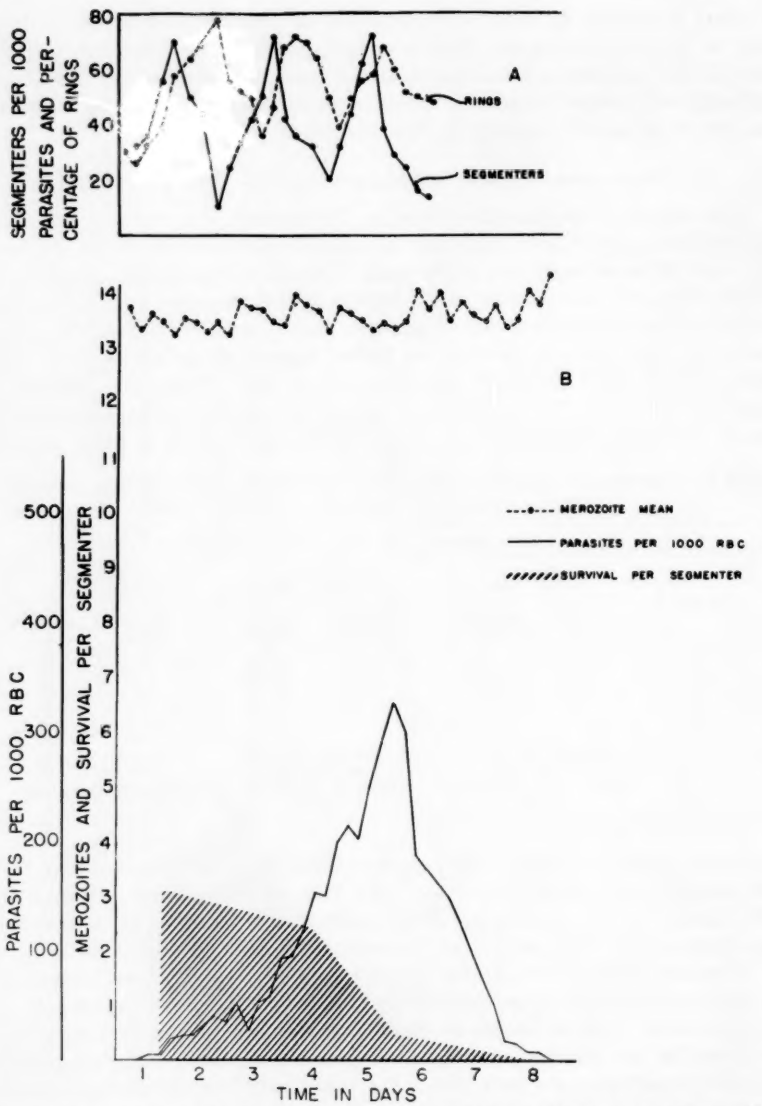

FIGURE 2. Course of infection, merozoite mean, survival and death per segmenter (Section B) and peaks of mature segmenters and "rings" (Section A) of *P. lophurae* developing in pheasants.

nmnity are indicated by (1) the high death rate during the acute rise, and (2) the unchanging merozoite mean throughout the infection; while the effects of acquired immunity are indicated by (1) an increased death rate at the crisis, and (2) the very rapid removal of parasites in superimposed infections.

III. *Plasmodium lophurae* in the American coot (*Fulica americana*)

Injection of massive doses and rapid passage were relied upon to establish *P. lophurae* in adult coots. As soon as a coot which had received a large dose of parasites from a chicken became positive, blood from it was injected into another coot. Initial infections were established according to the following transfers: from chicken to coot 37, from 37 to 38, to 47, etc. The first coot (37) never actually developed what could be called a frank infection, while the second (38) developed a parasitemia with a peak of only 355 parasites per 10,000 red blood cells and a merozoite mean of 14.24, although the merozoite mean of the original donor chicken was only 11.52. The third coot (47) and all subsequently infected coots developed rapidly rising fatal infections.

The development of *P. lophurae* was studied in 20 adult coots kept under natural conditions of night and day. Under these conditions the peaks of mature segmenters were on the average less than 36 hours, occurring regularly every 32 hours in some coots and varying slightly in others. Infrequent time lags in schizogony never amounting to more than 6 hours occurred, if at all, between the second and third segmentations. Varying from 6,000 to 13,280 the average peak of parasitemia in all coots studied (excluding coots 37 and 38) was 10,113 parasites per 10,000 red blood cells. Crisis forms characterized the infection just before and following the peak of parasitemia. Mitotic medium-sized and small lymphocytes and macrophages filled with parasites and malarial pigment are common in the blood at this time.

In coot 48 the peak of parasitemia was 9,160 per 10,000 red blood cells. The merozoite mean was $15.94 \pm .07$ ranging from 8 to 24, with 42% of segmenters containing 16 merozoites; 30% containing 14, and 19% containing more than 16 merozoites. Of the first segmentation when the merozoite mean was 14.90, 11.25 merozoites per segmenter lived, or 3.65 died; of the second segmentation when the merozoite mean was 15.10, 12.30 died or only 2.8 lived; and of the third segmentation when 16.94 merozoites per segmenter were being produced, less than 1 merozoite per segmenter or only .5 lived. In coot 49 segmentation regularly occurred every 32 hours, and the merozoite mean was $15.85 \pm .13$, ranging from 5 to 36. Of the first segmentation more than 80% of the merozoites lived. The peak of parasitemia in this coot was 13,280 parasites per 10,000 red blood cells. Figure 3 shows parasitemia, merozoite mean, survival and death of *P. lophurae* in coot 47, where development was somewhat irregular in that the reproducing parasites formed 2 broods segmenting 12 hours apart with each brood reproducing every 36 hours. Representing the highest death and the lowest survival rate in all coots studied, only 65% or about 9 of the 13.90 merozoites per schizont produced by the first segmentation lived in this coot.

About a month after their initial infections the only survivors, coots 37 and 38, were superinfected. In coot 37 the infection lasted about 4 days, with a peak of parasitemia reaching 1,260 parasites per 10,000 red blod cells, and a merozoite mean of 14.45. The time between the first and second segmentations was 32 hours, while that between the second and third was 40. Of the 14.40 merozoites per segmenter being produced during the first segmentation only 2.76 lived or 11.64 died. Of the second segmentation when 14.73 merozoites per segmenter were being produced

only .2 lived or 14.53 died. Thus the very slight initial infection of coot 37 produced parasiticidal antibodies strong enough to destroy about 80% of the merozoites produced by the first segmentation and practically all of the second.

Although there is some variation, development, survival and death rates of *P. lophurae* follow a definite pattern in coots, where reproduction and survival rates are invariably high and the death rate is always low.

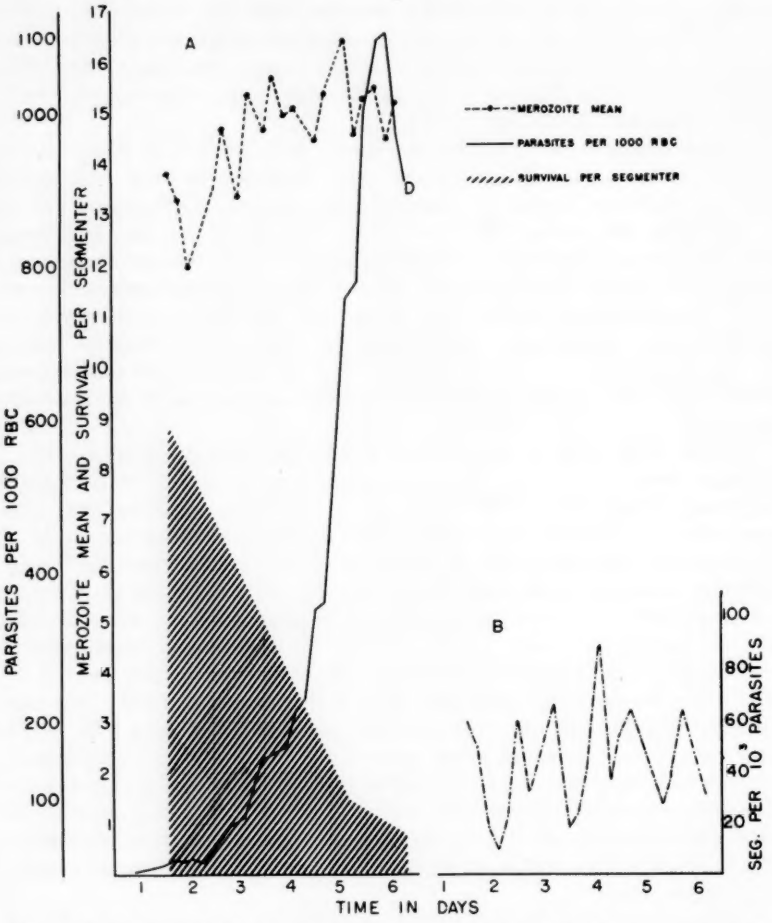

FIGURE 3. Course of infection, merozoite mean, survival and death per segmenter of *P. lophurae* developing in coot 47.

IV. *Plasmodium lophurae* in pigeons (*Columba livia*)

Plasmodium lophurae was studied in 20 wild-caught and in 8 domestic (pedigreed White Kings) pigeons kept on a 12-hour light and a 12-hour dark schedule. Although there appeared to be less synchronism in the wild-caught pigeons, the length of the asexual cycle was about the same in both groups. In general, para-

sitemia was higher in the White Kings than in the wild-caught pigeons and the former also showed symptoms, while the wild-caught variety never showed any outward signs of the disease.

Of more than 5,000 merozoite groups examined the merozoite mean was $11.05 \pm .05$, ranging from 4 to 18; 30% of these were composed of 12 merozoites; 60% were below 12, and only 10% contained more than 12 merozoites. In this one factor of reproduction rate, it was almost the same as that of the chicken ($11.05 \pm .05$ as compared to $11.71 \pm .02$ in the chicken), however the length of the asexual cycle in the pigeon, varying from 48 to 60 hours, greatly decreased the rate of reproduction in this host.

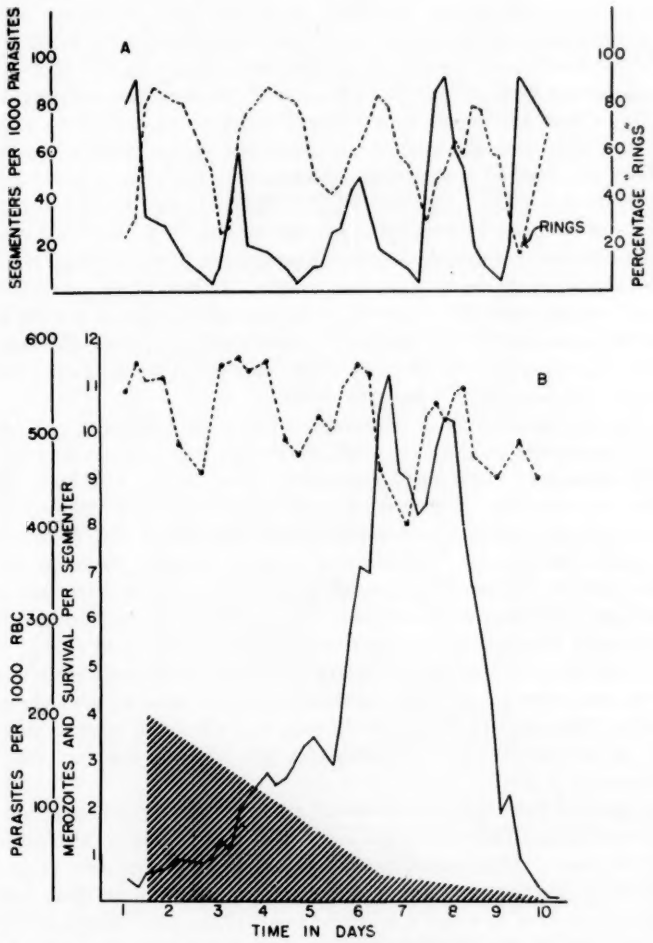

FIGURE 4. Development of *P. lophurae* in wild-caught domestic pigeons. Section A: peaks of segmenting forms represented by a solid line. Section B: merozoite mean is represented by a broken line; survival per segmenter by the lined area; death of merozoites per segmenter by the clear area between the merozoite mean and lined area; and parasitemia by a solid line.

Figure 4, Section A, shows the peaks of mature segmenters and "rings." The time between the peaks of segmentations 3, 4 and 5 is less than that at the beginning of the infection, the third peak coinciding almost exactly with the crisis. In Figure 4, Section B, we note a very great regular fluctuation in the merozoite mean, represented by the broken line, with the highest means coinciding with the peaks of greatest segmentation. The decrease in reproduction due to fluctuations in merozoite means of this sort amounts to about 15%.

DISCUSSION AND SUMMARY

The data obtained from blood-induced infections of *P. lophurae* in various avian hosts reveal some outstanding variations in the rate of reproduction and survival of parasites regulated by seemingly specific conditions within the hosts themselves. A partial explanation for these findings has been sought in the light of previous studies carried out by G. H. Boyd (1939) with *P. cathemerium* developing in a single species of host, the female canary; by Taliaferro and Taliaferro (1934, 1944, 1947, 1949, 1950), who have studied both avian and simian malaria, and by Thompson (1944) who studied host-parasite combinations of *P. mexicanum* and various lizards. Huff *et al* (1947) encountered a variability in morphology of pre-erythrocytic stages of *P. lophurae* developing in chickens, ducks, turkeys and guinea fowl. They are of the opinion that the degenerative changes in morphology resulted from adverse reactions of the hosts upon the parasite. Jeffery (1944) believed that the reaction of various susceptible hosts to sporozoites of *P. lophurae* is a variable regulated for the most part by the vertebrate hosts involved. Some differences in the reproduction and morphology of *P. relictum* were noted by Wolfson (1939) when she injected this parasite into domestic ducks.

The rate of reproduction of plasmodia depends on 2 factors: (1) the average number of merozoites produced by each schizont at segmentation and (2) the frequency of schizogony. Obviously a change in either factor or in both, will change the rate of reproduction. Information synthesized from data obtained from avian hosts studied in this series of experiments shows that one or both factors may vary. Comparing the behavior of *P. lophurae* developing in coots, pheasants and pigeons to that in chickens, the following general variations were noted: In coots the length of the asexual cycle was shortened (less than 36 hours on the average), while the average number of merozoites per schizont was increased ($15.43 \pm .11$); in pigeons the length of the cycle was greatly increased (about 56 hours), while the average number of merozoites per schizont remained about the same as in chickens ($11.05 \pm .05$); and in pheasants the length of the cycle was about the same as that in chickens, but the average number of merozoites per schizont was increased ($13.77 \pm .06$) (Figures 1, 2, 4, 6).

Data obtained from these experimental groups of birds emphasize that the basic rates of reproduction, destruction and survival of *P. lophurae* are determined by the nature of the hosts. Since plasticity and lack of host specificity seem to be outstanding qualities of this parasite, appropriate descriptions should be made only in conjunction with the host in which it is developing. These observations further emphasize the complex relations existing between host and parasite and the necessity of considering them together as a biological whole.

In chickens and pheasants parasiticidial effects of innate immunity are fairly uniform during the acute rise of the infection. During this time, on the average,

chickens destroy about 65% of the merozoites produced by each segmentation, and pheasants destroy 75% to 80%. In both hosts acquired immunity re-inforces innate immunity, resulting in a very rapid increase in destruction rates and a consequent drop in parasitemia (as the lined areas, representing survival of parasites, in

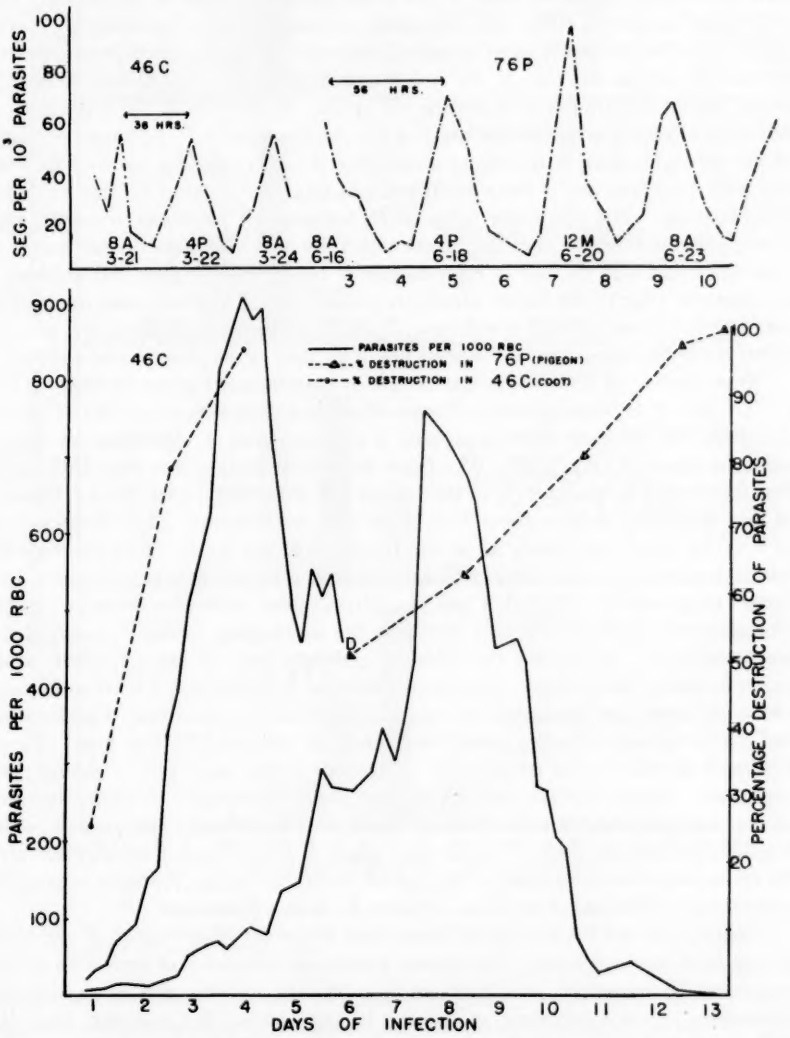

FIGURE 5. Comparison of the course of infection, length of cycle, percentage destruction of merozoites of *P. lophurae* developing in coots (46C) and in pigeons (76P).

Figs. 1 and 2 show). The decline of reproduction in chickens (Fig. 1) and the unchanging merozoite mean in pheasants and coots (Figs. 2 and 3) are interpreted to be due to relative amounts of parasiticidal substances in acquired immunity of these hosts. In the chicken-*lophurae* combination the parasiticidal substances are

not strong enough to kill the reproducing parasites and simply decrease their reproduction, whereas in the other hosts they are sufficiently strong to kill the parasites, and those which do live to reproduce produce merozoite groups characteristic of that host. The ability to destroy the progeny of the first and second segmentations and to some extent the third, is due to the innate immunity of the chicken (Taliaferro and Taliaferro, 1950), and apparently the same is true of pheasants. To this condition in the chicken is added acquired immunity resulting in a depression of reproduction and an increase of the powers of destruction of parasites; hence the abrupt decline in parasitemia following the crisis. In addition to the reproduction-inhibiting effects of acquired immunity of the chicken against *P. lophurae*, indicated by the noticeable drop in merozoite mean (Fig. 1), there was an increase in death rate after the crisis and a decrease in reproduction of 20% to 30% due to delays in schizogony. The merozoite mean of *P. lophurae* in pheasants remained high throughout the infection, but the innate immunity was also high in this host. In view of the fact that the rate of reproduction is considerably higher and uniform in the pheasant (due to the higher merozoite mean) than in chickens, and the peak of parasitemia is lower (9,373 in chickens; 3,250 in pheasants), it seems that the real difference in the immunities of the hosts lies in the titer of the parasiticidal antibodies.

Reproduction of *P. lophurae* was lowest in pigeons and highest in coots (Table I). In coots *P. lophurae* produces 3 segmentations with a merozoite mean of $15.43 \pm .11$, while for the same time in pigeons it produces only 2 segmentations with a merozoite mean of only $11.05 \pm .05$. Coots in general destroy less than 25% of the first segmentation, nearly 80% of the second and over 98% of the third. Pigeons, on the other hand, destroy about 50% of the first segmentation, 75% of the second, 83% of the third, and nearly all of the fourth, fifth and sixth. And although the rate of destruction is much slower in the pigeon than in the coot, in both hosts it increases progressively (Figs. 3, 4 and 5). Parasiticidal antibodies developed by the coot against *P. lophurae* are very strong as the unchanging merozoite mean in this host points out. In pigeons the infection generally ends in latency, while in the coot it is almost always fatal. In coots the increase in frequency of schizogony, high merozoite mean, and low innate immunity account for the rapid rise in parasitemia, and are correlated with the pathogenic condition produced in this host. Figure 4 shows a decrease in the frequency of schizogony in the late stages of the infection in pigeons. This is just the opposite of what would be expected to occur, inasmuch as acquired immunity has the tendency to depress metabolism and growth, which lengthens the asexual cycle. The fact that peaks 3, 4 and 5 are closer together after the crisis indicates an increase in the titer of antibodies so that the more susceptible parasites are killed and those left to segment do so at a faster rate.

Figure 6 shows the merozoite means and frequency distribution of merozoite groups in the various hosts. The highest percentage frequency of merozoite groups containing 16 merozoites is obtained in the coot. In chickens and in pigeons it is uncommon to find a segmenter containing 16 merozoites. We note that there is a shift downward in percentage frequency from 16-merozoite groups in coots, 14-merozoite groups in pheasants and 12-merozoite groups in chickens and pigeons with a concomitant decrease in the respective merozoite means of these hosts.

Although the behavior of *P. lophurae* in various avian hosts does indicate the effects of the host upon some of the fundamental aspects of the reproduction of the par-

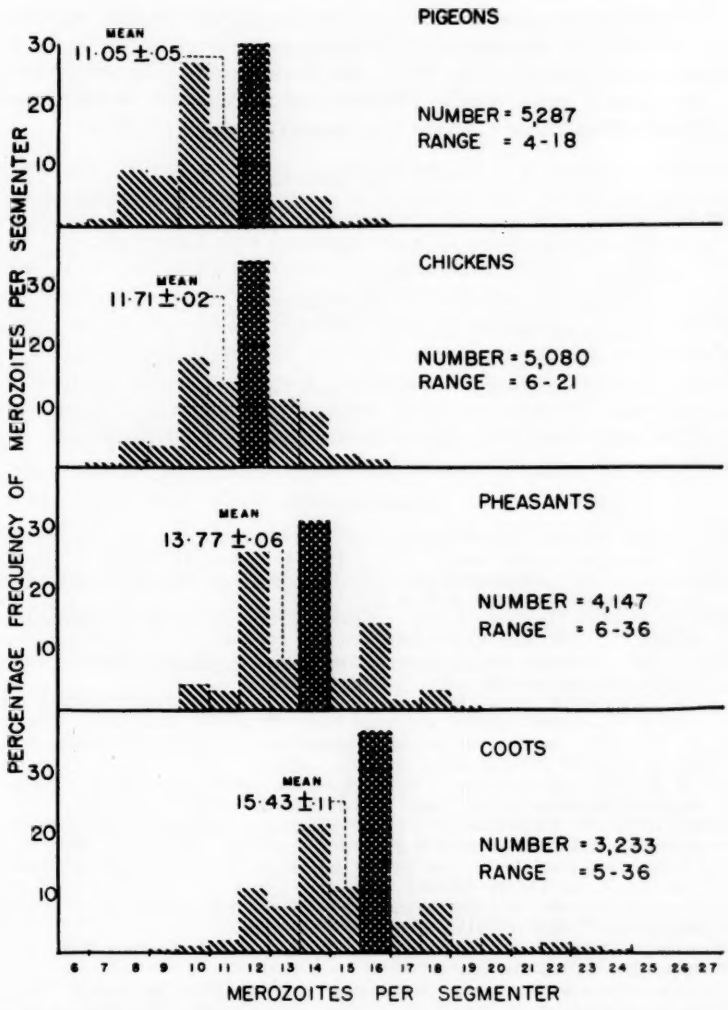

FIGURE 6. The percentage frequency of merozoite groups and merozoite means produced by various hosts infected with *P. lophurae*. Cross-hatched columns represent the highest percentage frequency of merozoite groups.

asite, a more precise study of this sort should originate from a single oöcyst so that more information concerning the genetics of the parasite would be available. Although blood-induced infections may be made up of a heterozygous population and would, therefore, be expected to react variously to the same conditions within a host, this sort of study does illustrate the effects of avian species upon the nature of the infection. The variations in reproduction recorded in this series of experiments do not seem to be due to changes brought about in the parasite, since at intervals it was

taken from various hosts and put back into chickens where the rates of reproduction and survival of *P. lophurae* are well known, as has been shown by the works of Taliaferro and Taliaferro (1940, 1950), and the characteristic chicken-*P. lophurae* rate of reproduction was restored. The study continued over a period of about 4 years and the infections were not made in sequence.

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DEVELOPMENT *IN VITRO* OF ISOLATED OOCYSTS OF *PLASMODIUM RELICTUM**

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Since 1947, the senior author has been carrying on a series of experiments designed to bring about the complete development *in vitro* of the mosquito phase of the avian plasmodium, *Plasmodium relictum.* The results attained have been published in a series of papers (Ball, 1947, 1948, 1954; Clark and Ball, 1952, 1954, 1956).

In the most recent report dealing with the development of the oocysts of this parasite while they were still attached to the stomach of the mosquito host *Culex tarsalis,* the oocysts increased in diameter up to threefold as compared to their original size. They ceased growing after 4 or 5 days in culture, although cytological staining showed in some cases no degenerative changes in the oocysts for as long as 3 weeks (Ball, 1954).

The mosquito gut continued contracting *in vitro* for as long as 5 weeks. However, histological study of the cultured mosquito stomach showed considerable degeneration of the epithelial layer even though the contractile tissue continued to function for some time.

The discovery by Weathersby (1952) that the mosquito stomach was not essential for the development of *P. gallinaceum in vivo* led us to attempt cultivation of oocysts dissected away from the stomach wall. This type of culture offers the opportunity of concentrating attention on the conditions necessary for the development of the plasmodium and does not present the additional problem of attempting to keep adult insect tissue alive and normal at the same time.

MATERIALS AND METHODS

Mosquitoes were dissected under aseptic conditions and the oocysts removed so that no contamination with micro-organisms from the lumen of the mosquito gut occurred. The technique employed has been described by Chao (1955). By sealing the mouth and anal openings of the mosquito with Duco cement and then sterilizing the outside of the insect, it was possible to obtain bits of the stomach wall with attached oocysts in a sterile condition. The oocysts, either free or attached to a piece of the stomach wall, were transferred whenever necessary by means of a tiny piece of broken No. 1 coverslip, which served as a kind of "ferry." The oocysts were cultured either in sealed hanging drops or in Carrel flasks. In the latter instance, they were gassed continuously with a mixture of 5% CO_2-95% air by means of a cotton-stoppered tube inserted through a cotton plug in the neck of the flask. Such an attachment could easily be removed in changing the medium. The cultures were rocked up and down at a frequency of 7 times a minute through a 25° arc. Transfers to fresh medium were made ordinarily every 48 hours, occasionally every 24 or 72 hours. An inverted microscope proved especially useful for examining cultures in Carrel flasks. All cultures were kept at 21°–22° C; ordinarily after an initial 24-hour period at 18° C (Ball, 1954).

The types of media used were in general those employed in previous experiments in which the oocysts were cultured while attached to the stomach of the mosquito. The constituents may be grouped roughly into (1) organic constituents of known chemical composition, (2) inorganic salts at a concentration found to exist in insect tissue (Clark and Ball, 1954) or else discovered to be suitable as a physiological salt solution for insect tissues, (3) chicken serum, and (4) chick embryo extract. Tables I and II give the constitution and proportions of the successful media used. The proportions and to some extent the ingredients were varied with the

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aim of producing such improvements in culture as would be evidenced by increase in size and/or continued internal development culminating in the formation of infective sporozoites. Numerous other modifications of the media were tried, but only those which resulted in the growth of the oocysts are given here.

The inorganic constituents of media A through G are listed at the end of Table I. Medium B differed from Medium A by the omission of the amino acids. Medium C had a lower concentration of chicken serum. Medium D had twice the concentration of chicken serum of Medium C, but a lower concentration of inorganic salts. Penicillin at a concentration of 600 units per ml and streptomycin at 200 units per ml were added to media A through D just before use.

In media E, F, and G, the organic and inorganic constituents were somewhat different in

TABLE I.—*Composition of synthetic portion of media A & C, E & F for cultivation of oocysts of* Plasmodium relictum

Material	mg/l A&C	mg/l E&F	Material	mg/l A&C	mg/l E&F
DL-Alanine	40	—	Co-carboxylase	4	—
L-Arginine-HCL	56	24	Folic acid	8	0.01
DL-Aspartic acid	48	—	Niacin	8	0.06
L-Cystine	16	19	Niacinamide	8	—
L-Glutamic acid	789	—	*p*-Amino benzoic acid	0.46	—
L-Glutamine	464	36	Pyridoxine-HCL	4	0.06
Glycine	40	—	Riboflavin	4	0.03
L-Histidine-HCL	16	10	Thiamine-HCL	8	0.6
L-Isoleucine	32	31	Adenine sulphate	3.2	1.7
L-Leucine	96	31	Cytosine	3.2	1.7
L-Lycine-HCL	56	36	Guanine	3.2	1.7
DL-Methionine	24	20	Hypoxanthine	1.6	1.7
L-Phenylalanine	40	22	Thymine	3.2	1.7
DL-Proline	32	—	Uracil	3.2	1.7
DL-Serine	48	—	Xanthine	3.2	1.7
DL-Threonine	48	58	Sodium Acetate	1160	—
DL-Tryptophane	16	14	Succinic acid	1390	—
L-Tyrosine	—	24	NaCl	7500	8430
DL-Valine	40	58	KCl	350	240
DL-α-Amino butyric acid	80	—	$CaCl_2$	210	—
Glutathione	0.8	—	$Ca(NO_3)_2\ 4H_2O$	—	310
Glucose	11600	6020	$Mg\ Cl_2\ 6H_2O$	—	120
Fructose	928	—	$NaHCO_3$	100	2700
Ribose	4	—	Na_2HPO_4	—	270
Ascorbic acid	4	—	KH_2PO_4	—	120
Biotin	0.008	0.01			
Ca pantothenate	4	0.06			
Cholesterol	1.9	—			
Choline	4	—			

TABLE II.—*Relative proportions (given in milliliters) of main constituents of medium*

	Organic	Inorganic	Chicken Serum	Chick Embryo Extract
Medium A	1.0	1.0	1.0	1.0
Medium B	1.0 (Minus amino acids)	1.0	1.0	1.0
Medium C	1.0	1.0	0.4	1.0
Medium D	1.0	0.75	0.8	1.0
Medium E	4.0	1.0	1.2	0.5
Medium F	4.0	1.0	1.7	0.5
Medium G	1.0 (Glucose only)	1.0	8.0	4.0

composition and strength from those of media used previously. In this series, the results of Waymouth (1955) and of Eagle (1955) in formulating successful culture media for the growth of chick and mammalian tissue were combined with our own findings of the constituents essential for the growth and maintenance of oocysts. The relative amounts of the four constituents of the final medium are given in Table II. Medium G consisted only of glucose, inorganic salts, chicken serum and chick embryo extract. No antibiotics were used with media E, F, or G.

RESULTS

In many attempts, no or very slight increase in size occurred in the cultures of oocysts either when they had been completely isolated from the stomach or else when they still remained attached to shreds of stomach wall.

In other instances, however, the oocysts continued to increase in size up to a maximum at 5 to 8 days' cultivation, but then failed to develop further (Table III).

In a few cases, increases in size occurred more gradually and the maximum was not attained until the 10th or 11th day. In no instance, however, did development proceed to the sporozoite stage, but slides of the oocysts from culture showed that they had undergone some development during the period of cultivation. The increase in size of the oocysts in favorable cultures did not equal the greatest relative increase in size found to take place for oocysts which remained attached to the whole stomach of the mosquito (Ball, 1954), although the oocysts in general continued to grow over a greater number of days.

Some combinations resulted in greater size increases than did others. Although regular increase in size occurs in sealed hanging drops changed every 24 or 48 hours, Carrel flask cultures with greater volume of medium (1.5 ml *vs* 0.03 ml) and controlled aeration showed a relatively greater size increase (Table III). On the

TABLE III — *P. relictum* - *Size of oocysts in culture*

Medium and Type of Container	Date Started	Oocyst size - cross section area in μ^2 Days cultured														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A - H.D.	3/17/55	756		1200*		1050*	980									
A - H.D.	"	616		990*		1050*	1200*	1300*		1120						
A - H.D.	"	750		1575*		1020*	1330	1295*		1330						
		700		1225*		840*	840									
A - H.D.	"	700		1600*		1225*	1440	1440*		1295						
B - H.D.	3/19/55	1050		1140*	1330	1440*		1225*								
B - H.D.	"	1400		2750*	2750	2250*		2600								
B - H.D.	"	1400		2750*	2250	2600*		2250								
C - H.D.	3/24/55	840		1800*		1295*		1140*		1400			875*	1295		1480
C - H.D.	"	644		1800*		1400*		1225*		1050			1225*	1050		1120
C - H.D.	"	900		1440*		1520*		1440*		1470			1520*	1600		1155
C - H.D.	"	750		900*		1140*		756*		1225						
D - H.D.	4/18/55			*		3250*			3300*		4500*		3500			
D - H.D.	"			*		2340*			2500*		4320*		5760			
D - H.D.	4/21/55	2160	2750*			2750*		3025								
E - H.D.	1/13/56		1800		1800		2160*		2750			2750*				2750†
F - C.F.	4/13/56				1225*		1681*		2726							
G - C.F.	6/13/56		1296	1404*		2303*		2773								
G - C.F.	"		1225	1369*				2209								

* Transferred
† No change in size for next 38 days.

other hand, a fairly high proportion of Carrel flask cultures showed no development of the oocysts.

Apparently chicken serum and embryo extract contain most of the substances necessary for partial growth of the oocysts, since these developed in Medium G, which had only glucose and inorganic salts added to the serum and the embryo extract. With the complex synthetic medium, the concentration of chicken serum could be reduced (medium C) but oocyst growth did not occur if it was completely eliminated.

SUMMARY

From the results described here, it has been shown that the oocysts of *P. relictum* may develop *in vitro* apart from the intact stomach of the mosquito vector, but development does not proceed beyond stages reached by the oocysts attached to an entire stomach which has not been injured. This work confirms that of Weathersby (1952) who showed by injection of gametocytes into the haemocoele of *Aedes aegypti* that the mosquito stomach is not required for development of *P. gallinaceum*. On the other hand, we have not yet been successful in bringing about complete development *in vitro* of the oocysts of *P. relictum* either attached to the whole mosquito stomach or dissected away from the stomach wall.

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RESEARCH NOTE

FAILURE TO TRANSMIT MOUSE LEUKEMIA BY *TRICHINELLA*

Successful transmission of viral agents by helminthic parasites has previously been reported. (Shope, 1941, J. Exp. Med. **74**: 49–68; Syverton *et al*, 1947, J. Exp. Med. **85**: 759–769). Because it is believed that this mode of transmission may play an important role in the etiology of viral infections, similar studies have been undertaken in this laboratory. Our attention has recently been directed to experimental passage of mouse leukemia by the nematode, *Trichinella spiralis*, since it has been postulated that this condition, which develops "spontanously" in certain strains of mice, is produced by a filterable agent to which have been attributed virus-like properties (Gross, 1952, Ann. N. Y. Acad. Sci. **54**: 1184–1196).

Three experiments, each with slightly modified procedure, were carried out using young AKR mice 5½ to 8 weeks old. The initial inoculum used in all experiments consisted of 0.1 ml of a 20% tissue suspension introduced intraperitoneally. Such suspensions uniformly produced leukemia in 9–14 days in experimental animals.

In the first instance, 2 lots of mice received 0.1 ml of leukemic suspension intraperitoneally on the 6th and 22nd days respectively, following oral administration of 300 *T. spiralis* larvae. In the second experiment, 3 groups of mice received 0.1 ml of suspension 5, 11, and 15 days respectively after oral administration of 250 *T. spiralis* larvae. In the final experiment 3 lots of mice were given 275 larvae orally on days 0, 5, and 10; 5 days following administration of larvae to the 3rd group, all 3 groups were inoculated with 0.1 ml of suspension of leukemic tissue.

All mice were examined daily and, in total 24 were sacrificed when signs of acute leukemia became evident. *T. spiralis* larvae were recovered from these leukemic mice by peptic or tryptic digestion of infected muscle tissue and thoroughly washed in sterile isotonic salince. Three mice were subinoculated with *T. spiralis* by oral administration of 200, 300, and 375 larvae respectively, while 23 mice received suspensions consisting of from 400 to 2000 thoroughly ground larvae per mouse. These subinoculated mice were observed for 60 days but in no instance were any signs or symptoms of leukemia detected. Attempts to transmit leukemia to suckling mice by use of the same technics are in progress. (This investigation was supported by research grant E1198 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service. We are indebted to Dr. Anna Dean Dulaney for stocks of AKR and leukemic-carrier mice.)—M. ELOISE ROWLAND AND ROBERT C. RENDTORFF, *Division of Preventive Medicine, The University of Tennessee, College of Medicine, Memphis, Tennessee.*

PARASITEMIA AND SURVIVAL IN INBRED STRAINS OF MICE INFECTED WITH *PLASMODIUM BERGHEI*

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Inbred strains of mice were found to fall into 4, or possibly 5, categories according to how long they survived after infection with *Plasmodium berghei* (Greenberg, Nadel, and Coatney, 1953, 1954). In most of the strains of mice used, survival was bimodal, with a peak of deaths at the end of the first week and another at the end of the second or third week (Nadel, Greenberg, Jay, and Coatney, 1955). Mean survival appeared to be an expression of the relative numbers of mice which survived the first wave of death. The bimodal death curve was paralleled by pathological changes observed in the mice (Highman, Greenberg, and Coatney, 1955). At the end of the first week, in both long- and short-lived mice there were pathological changes, such as fatty infiltration of the heart muscle, which disappeared during the second week. Pathological signs suggestive of anoxic anemia began to appear at the beginning of the third week. Furthermore, deaths at the end of the first week were coincidental with two other events: (1) a crisis in the parasitic infection of mature erythrocytes and (2) a rapid drop in total erythrocytes (Greenberg and Coatney, 1954, Greenberg 1955). Following these events young erythrocytes made up most of the population of erythrocytes; these were infected to the extent of over 90%. No crisis, corresponding to that observed in parasitemia of mature erythrocytes, has been observed in infected immature erythrocytes. The parasitemia itself, therefore, was bimodal, being predominantly in mature erythrocytes during the first week and mostly in immature erythrocytes after the first week.

It seemed probable that the first wave of death in mice was caused by the crisis of the infection in mature erythrocytes and the acute hemolysis. Mice with different genetic backgrounds appeared to differ in the percentage of mature erythrocytes infected at the peak of the infection (Greenberg and Coatney, 1954, Greenberg, 1955). In the 4 genetic variants of mice studied, there seemed to be a rough inverse correlation between the maximum parasitemia of mature erythrocytes and length of survival. Stated generally, this hypothesis says: The higher the parasitemia of mature erythrocytes during the first week, the less likely the mouse is to survive beyond the first week. As a corollary to this, there was the hypothesis that the degree of parasitemia of mature erythrocytes was characteristic of each strain of mouse and was presumably a function of heritable characters. Resistance to malaria in terms of survival would then be a simple function of the inherited ability of the mice to control the infection in mature erythrocytes during the first week after inoculation.

These observations and the derived hypotheses have now been examined in larger series of inbred mice as well as in noninbred Swiss mice.

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METHODS

The Kasapa strain of *P. berghei,* maintained by weekly subpassage in white Swiss mice, was used throughout. All mice were inoculated with 1 million parasitized erythrocytes via a tail vein. Almost all were approximately 2 months old when inoculated.

The mice were generously supplied by Dr. George E. Jay, Jr., of the Animal Production Section of the National Institutes of Health.

Blood smears were made on day 6 and examined, after staining with Giemsa's stain, for percentage of mature erythrocytes infected. The method of counting parasites was such that the lower the parasitemia the larger the number of fields examined, so that the expected error in counting was less than 10%. In early experiments, parasite counts were made daily on each mouse until it died. This was burdensome and yielded very little additional useful data that could be used for intra-strain comparisons. Counts made earlier than day 6 were in some strains not very reliable because the counts were too low. Counts made later than day 6 were of little use for comparative purposes because in some strains many of the mice failed to survive beyond the sixth day. The crisis in the infection occurred later than day 6, so that observations taken on that day could be taken as a reasonable measure of the rate of increase in parasitemia from the time of inoculation of parasites.

Though there was a definite purpose in performing the present experiments, much of the early work was exploratory. Small numbers of mice of many strains were tested from week to week to determine their suitability in terms of intra-strain differences and similarities. There was the additional consideration of availability. Some strains were rejected because they were rather difficult to obtain regularly in sufficient numbers. STR/N on the other hand, which was also difficult to obtain, was retained in the series because it reacted differently to the infection than did other strains. Other strains used were A/LN, DBA/2JN, C57Bl/6JN, BALB/CANN, C_3H/Hen.

There was, then, an early exploratory period when differences were being sought and a later period when an attempt was made to confirm or refute these observed differences with large numbers of animals and more trials. There was a limit to the number of mice which could be inoculated at one time from the same inoculum. This limitation precluded any large-scale experiments using many mice of each of the several strains which appeared worth investigating.

While the goal, an arbitrary one established part way through the experiment, was 100 mice of each strain, it became obvious that the variance from week to week was much larger than could be accounted for by simple animal variance. It became more important, then, to accumulate the results of many different trials over a long period of time. The experimental design of choice was to have a number of trials of each strain, using as many different strains as possible at the same time, and employing small numbers of mice (5 or 6 of the same sex) per trial. In practice all strains were not tested concurrently (except that white Swiss mice were used in every test).

The mean sixth day parasitemia in mature erythrocytes for each strain was calculated from group means, so that even though over 100 mice of each strain were used, the effective sample size of each strain was considerably smaller.

RESULTS

Table I summarizes the results of observations made on the sixth day parasitemia of mature erythrocytes. When there were sufficient trials to make valid comparisons, females had significantly higher counts than males. This was true of C57Bl, A, and BALB mice. STR males had lower counts than the females but the sample was too small to be significant. On the other hand, the small number of groups of male C_3H and DBA mice had counts slightly higher than the females. For the purpose of this paper the discussion of parasitemia will be confined to the females (except for STR) which are more adequately represented.

The parasitemia of female Swiss mice was significantly higher than that of all other strains of mice, and that of STR was significantly lower. There were no significant differences among BALB, C57Bl, A, or C_3H mice, but DBA mice appeared to be intermediate between this group of strains and Swiss.

The mortality data of all mice (male and female) are shown in Figures 1 and 2. It would be misleading to calculate mean survival because of the multimodal distribution of deaths in most of the strains. It is obvious that Swiss, A, and C_3H mice form a family of strains with similar mortality distributions. There is a decided peak of deaths at the end of the first week and a suggestion of another minor wave of deaths at the end of the second week. DBA mice appear closely related to this family but the small differences appear to be reasonably reproducible in large series to suggest that DBA mice may be slightly longer lived. The distribution of deaths in STR, BALB, and C57Bl appears to be unique in each strain. C57Bl mice were by far the longest lived of the strains because fewer mice died in the first wave of death and the second wave of death occurred about a week later than in the other strains. BALB mice presented another problem. Nineteen groups of these mice were inoculated. In 3 of these groups none of the animals survived the fifth day and in 3 other groups less than half the animals survived the fifth day. These 6 groups accounted for practically all the deaths at the end of the first week. Yet even including these groups of mice, the distribution of deaths in BALB mice is different from that of other strains used in this study.

TABLE I— *Mean sixth day percent infection of mature erythrocytes in several strains of mice*

Strain	Females		Males		All Mice	
	No. Trials	Mean ± Standard error	No. Trials	Mean ±	No. Trials	Mean ±
STR/N	11	5.1±0.6	2	3.5	20*	5.8±0.7
BALB/CANN	8	22.1±3.3	8	12.1±2.0	16	17.1±2.3
C57 B1/6JN	15	21.6±1.9	16	12.1±1.2	31	16.7±1.4
C_3H/Hen	13	24.5±2.5	4	28.0±4.8	17	25.3±2.2
A/LN	8	25.6±2.2	6	15.0±3.2	14	21.1±2.3
DBA/2JN	9	34.9±4.1	4	39.0±4.4	13	36.2±3.1
Swiss	29	49.3±2.5	1	49.0	30	49.3±2.5

* There were 7 groups of mixed males and females

Half of the A, Swiss, C_3H, and DBA mice were dead by day 6; half of the BALB and STR mice by day 12; and half of the C57Bl by day 22. By day 10

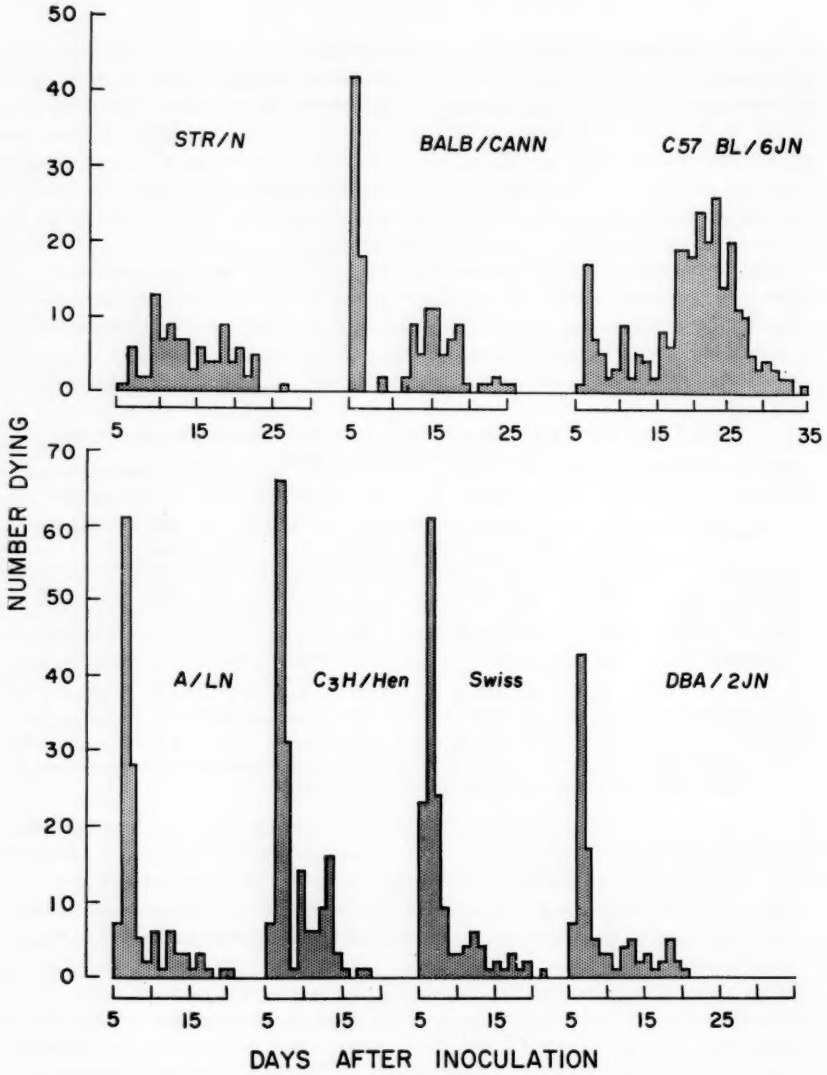

FIGURE 1. Mortality in mice of 7 strains infected with *P. berghei*.

between 64 and 74% of all A, Swiss, C_3H and DBA mice were dead; whereas only 49 percent of BALB, 31 percent of STR, and 13 percent of C57Bl mice were dead.

A comparison has been made (Table II), within strains, of the average parasitemia of mice dying before the tenth day and those dying after the tenth day. In every strain the counts in mice dying before the tenth day was 1.5 to 2.0 (average 1.8) times as high as in those dying after the tenth day.

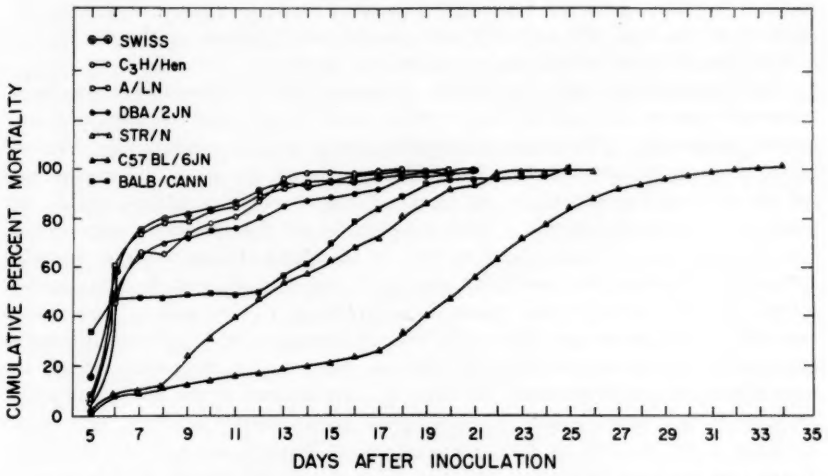

FIGURE. 2. Cumulative percent mortality in 7 strains of mice infected with *P. berghei*.

TABLE II — *Relationship between early and late deaths and sixth day parasitemia, all mice of several strains*

	Sixth day parasitemia in mice which survived	
Strain	10 or less days	11 or more days
Swiss	53.1	29.5
DBA	38.3	24.7
C_3H	27.3	18.4
A	22.9	12.0
BALB	25.4	13.6
C 57 B1	29.5	15.0
STR	8.3	4.7

DISCUSSION

It is obvious that each strain of mouse in the present study did not have a unique response to *P. berghei* as measured by sixth day parasitemia. Four of the strains resembled each other rather closely, A, C_3H, BALB, and C57Bl, with the possibility that DBA mice might also belong to this group, though their counts were on an average higher. On the other hand, Swiss mice had distinctly higher parasitemias than all of the other strains. This, it might be argued, can be the result of using a parasite which, through serial passage, was adapted to the Swiss mouse. On the other hand, the parasitemia of STR mice was significantly lower than that of all other mice in the series, and no such logical reason for this observation comes to mind immediately. It is suggested that genetically there are 4 different types of mice so far as their reaction to *P. berghei* is concerned: (1) Swiss, (2) DBA, (3) A, C_3H, BALB, and C57Bl and (4) STR. Resistance to malaria in

terms of infection of mature erythrocytes would then be a function of genetic constitution of the host, and certainly some simple observations on the infection in hybrids should reveal something of the genetics involved.

Superimposed on these apparently strain-associated differences were large, seemingly random fluctuations from week to week, which might properly be considered nongenetic. There was some evidence of a sex difference but no certainty as to whether the sex difference was the same in all strains. The explanation for the random fluctuations is not clear. Perhaps, as Bray (1954) states, the parasite is extremely variable. This, however, leaves the variability unexplained. On the other hand, it may be that the use of inbred strains actually increases rather than decreases the variability, similar to the variability in the responses of inbred strains of mice to diet reported by Grünberg (1954) and to barbiturates reported by McClaren and Michie (1954). Grünberg (1954) offered alternative explanations for the variability. For instance, even though intensively inbred, the mice might still not be perfectly homozygous. Or because of the poor "buffering" capacity of the inbred strains of mice, small environmental changes, even those applying to the mothers of the mice used, might produce exaggerated responses. If the last were an important explanation, then perhaps inbred strains of mice might be useful as amplifiers of small environmental changes, provided their sensitivity were not so great that static would interfere with interpretations. Whatever the explanation for the week-to-week variations, it is reasonably certain that the contributions made by the strain of mouse can be discerned, if a sufficiently large number of samples is taken over a period of time.

The influence of the infection of mature erythrocytes on survival is not entirely clear. Within each strain it is certain that mice which died early had higher counts than those which died late. However, when comparisons are made among strains, the relation between parasitemia and survival breaks down almost completely (Table III). It is impossible to predict, on the basis of the rate of increase

TABLE III.—*Strains of mice grouped by similarities in mean sixth day parasitemia and survival*

	Sixth day parasitemia		*Survival*
I.	Swiss (highest)	I.	Swiss, C3H, A, DBA (lowest)
II.	DBA	II.	STR
III.	C3H, A, BALB, C 57 Bl	III.	BALB
IV.	STR (lowest)	IV.	C 57 Bl (highest)

in parasitemia of mature erythrocytes during the first week, what percent of the mice of a given strain would survive the first wave of deaths.

Yet, there is a relation among events occurring at the end of the first week. At this time there is a crisis in the infection of mature erythrocytes, a rapid destruction of mature erythrocytes, a peak of certain pathological changes, and a major wave of deaths in almost every strain of mouse (Greenberg 1955). Death is probably associated with the hemolytic crisis, and its associated insults as reflected in pathological changes in the tissues. The relation between parasitemia and the hemolytic crisis is less clear, as was pointed out earlier (Greenberg and Coatney, 1954). The crisis in Swiss mice does not occur until 50% of the mature erythrocytes are invaded, but it occurs in STR mice when less than 10% of the cells are invaded. The crisis would appear to occur independently of the one tangible triggering mechanism, the number of erythrocytes invaded. Also not clear is the relation between the crisis, parasitic and hemolytic, and survival itself. From

earlier work (Greenberg, 1955) there appeared to be only minor differences among strains of mice as to when and how rapidly the hemolytic crisis occurred. There appeared to be only insignificant differences in severity of the resulting anemia. Yet 80 to 90% of the mice of certain strains survived the crisis whereas less than 25% of others survived it.

It would seem that there are two characteristics of *P. berghei* in mice which are dependent on the strain of mouse: (1) the degree of parasitemia developed during the first week and (2) the number of mice that died at the end of the first week. Mice vary in their ability to control the parasitemia and in their ability to withstand the insult of the infection. These appear to be two partially dependent variables apparently under separate genetic control. STR mice could be classified as low parasitemia, low tolerance mice, while C57Bl would be moderate parasitemia, high tolerance mice. Swiss mice would be high parasitemia, low tolerance mice.

Some fairly obvious experiments suggest themselves: (1) To determine whether the high parasitemias in Swiss are the result of adapting *P. berghei* to that strain of mouse, one could pass the parasite serially in one or more of the other strains and (2) to determine whether and how resistance to parasitemia is inherited, one could study the infection in hybrids of the extremes, Swiss and STR. These experiments are in progress and will be reported in a later paper.

SUMMARY

Mice of 6 strains; A/LN, DBA/2 JN, C_3H/Hen, C57Bl/6JN, BALB/CANN, STR/N, and Swiss, were examined on the sixth day after inoculation with *Plasmodium berghei* for the percentage of mature erythrocytes infected. Swiss mice had significantly higher counts than all other mice, and STR had significantly lower counts. Within strains, mice which died before the tenth day had counts which were almost twice as high as those which died after the tenth day. Among strains, however, there appeared to be little correlation between average sixth day parasitemia and overall survival. It was concluded that parasitemia and survival were two partially dependent variables, both of which were determined by the strain of mouse.

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SOME CHARACTERISTICS OF *PLASMODIUM BERGHEI* PASSED WITHIN INBRED STRAINS OF MICE

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It was shown in an earlier publication (Greenberg, 1955) that a crisis occurred in mice infected with *P. berghei* at the end of the first week. The crisis was confined to mature erythrocytes, which were rapidly removed from circulation. In mice of different genetic backgrounds there were differences in the percent of mature erythrocytes infected at the time the crisis occurred. More extensive observations were made on a series of 6 inbred strains of mice, and general-purpose white Swiss mice (Greenberg and Kendrick, 1956). When measured on the sixth day after inoculation, the infection of mature erythrocytes of Swiss mice (mean 49%) was significantly higher than that of the 6 inbred strains of mice. A, BALB, C 57 Bl, and C_3H mice had comparable infections (mean 20 to 25%) and DBA mice had counts (mean 35%) significantly higher than the last group of mice but lower than Swiss. STR mice had significantly lower counts (mean 5%) than all the other strains of mice.

It was concluded that the degree of infection of mature erythrocytes was a characteristic of the strain of mouse. On the other hand, if one ignores, temporarily, the results in STR and one assumes that, by chance, the observation in DBA was an extreme high, then all the strains of mice except Swiss could be classified in one group. In all the preceding experiments Swiss mice had been used as donors of infected blood, and *P. berghei* had been routinely passed in Swiss mice for several years. The use of mice of a common strain as a donor was not itself an unreasonable procedure, but it is possible that the extremely high parasitemias seen in Swiss mice could have been an expression of a Swiss mouse-adapted parasite. If this were true, it should be possible to demonstrate that the parasite can become adapted to all, or some, of the other strains of mice by serially passing it through these mice. One would expect that when and if the parasite adapted to other strains of mice, the parasitemia in these strains would be comparable with that in Swiss mice infected from Swiss donors. The results of this type of experiment in several strains of mice is the subject of the present paper.

MATERIALS AND METHODS

The following strains of mice were used: C_3H/Hen; STR/SN; A/LN; BALB/CANN; C 57 Bl/6JN; DBA/2N, and general-purpose white Swiss. The mice were all furnished by Dr. George E. Jay, Jr. of the National Institutes of Health.

The design of the experiments was as follows: Representatives of each strain of mouse (except STR which was done later) were inoculated at transfer O from a common white Swiss mouse. The parasites were then passed serially, weekly, and independently through each of the inbred strains of mice along with the line

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maintained in Swiss mice. Each mouse received 1 million parasitized erythrocytes through a tail vein. Eight to 10 mice were used in each serial transfer and 1 of these was killed on the seventh day and used as a donor for the next passage. Giemsa-stained blood smears (tail vein) were made on day 6 and examined for the percentage of mature erythrocytes infected. The survival time of each mouse was also noted. Again, except for STR mice, the parasite was transferred 21 times in each strain of mouse.

Most of the mice used were females, but there were a few groups of each strain composed of males. Data on these have not been included in calculating means. The number of groups of males used were: C_3H, 1; A, 3; BALB, 6; C 57 Bl, 3; and DBA, 4. In 1 passage through DBA, only 1 mouse was used. The mice were received as weanlings and inoculated when 2 months old, plus or minus 1 week.

Mean parasitemia and the standard errors were calculated from group means.

RESULTS

The mean sixth-day parasitemia, as percentage of infected mature erythrocytes, for each strain over all passages after passage O (females only), is shown in Table I. Swiss mice had counts significantly higher than those of all other mice ($p <$ 0.001). DBA counts were lower than Swiss but higher than those of all other mice ($p < 0.01$). With the exception of C_3H mice, the order and magnitude of parasitemia was the same in the present series as it was in the previous series when Swiss donors were used (Greenberg and Kendrick, 1956). Strain A mice had counts somewhat lower in the present series than in the previous series ($p <$ 0.01), as did STR. The difference between the mean parasitemia of these mice in the present series and the previous series was statistically significant ($p < 0.01$).

TABLE I — *Mean 6th day parasitemia (% mature erythrocytes infected) in 7 inbred strains of mice. (female)*

Mouse Strain	6th day parasitemia (mature erythrocytes)	
	Swiss donor*	Within-strain donor
Swiss (Swiss donors)	49.3 ± 2.5 (29)	46.7 ± 1.7 (21)
Swiss (C_3H donors)		18.8 ± 2.2 (16)
DBA/2N	34.9 ± 4.1 (9)	31.8 ± 2.7 (17)
C_3H/Hen	24.5 ± 2.5 (13)	3.4 ± 0.8 (19)
A/LN	25.6 ± 2.2 (8)	16.8 ± 1.2 (18)
C 57 B1/6JN	21.6 ± 1.9 (15)	20.5 ± 2.2 (18)
BALB/CANN	22.1 ± 2.4 (8)	20.3 ± 0.8 (15)
STR/N	5.0 ±	2.0 ± 0.6 (7)

Figures in parentheses are number of groups of mice used.
* From Greenberg and Kendrick, 1956.

It is possible that the differences observed in A mice in the 2 series may have been an artifact in spite of the statistical analysis for significance. In the present series the mean parasitemia during the first ten passages was 14.2% and during the last ten 19.3%. There was no evidence of a progressive or persistent trend toward

lower infections. Even if the differences between A mice of the 2 series were real it is relatively small (26% with Swiss donors, 17% with A donors).

With C_3H mice (Fig. 1) there was a gradual trend over the first 4 or 5 passages toward a lower parasitemia. By the sixth passage the average parasitemia for each group of mice had reached about 2% and has since persisted at about

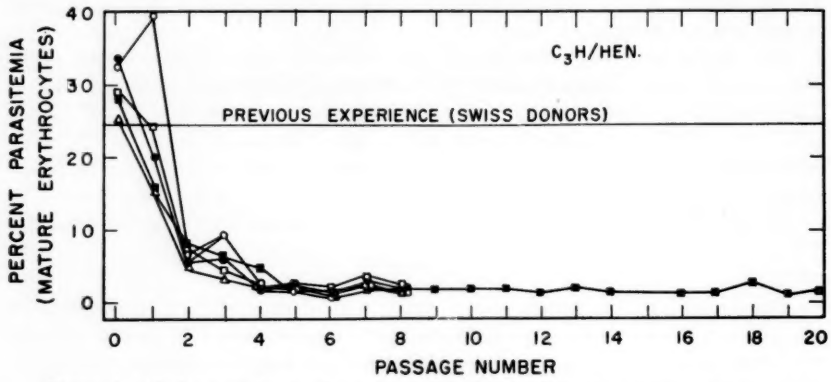

FIGURE 1. 6th day parasitemia of mature erythrocytes in C_3H mice during serial passage of *P. berghei* in C_3H mice.

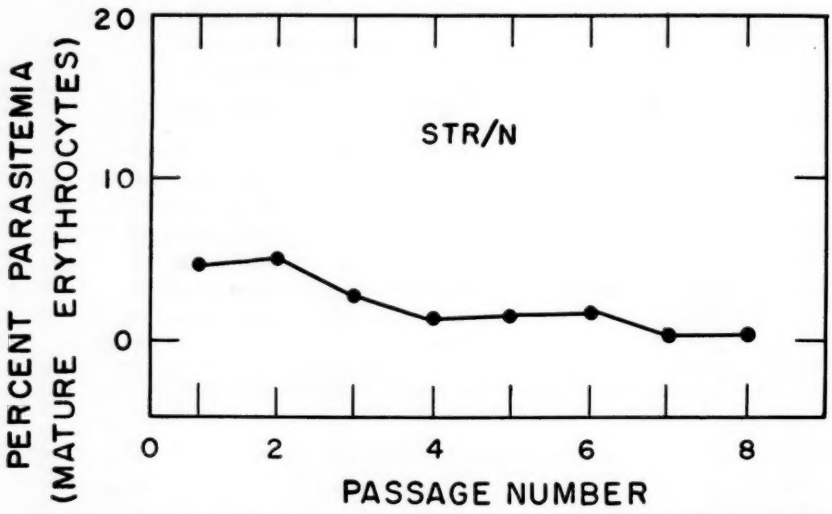

FIGURE 2. 6th day parasitemia of mature erythrocytes in STR mice during serial passage of *P. berghei* in STR mice.

this level for some 40 passages (not all shown on the figure). On the assumption that the observations on C_3H may have been accidental, the parasite was passed serially through C_3H mice in 4 additional independent series. These results are also shown in Figure 1. It is clear that with minor variations the parasitemia dropped at about the same rate in all 5 series and levelled off at about 2%.

STR mice, in a limited series, had a somewhat similar history to C_3H mice (Fig. 2).

The C_3H-passed parasites were reinoculated into Swiss mice in each of 16 almost consecutive passages beginning with the 12th C_3H passage. Sixth day counts in these Swiss mice averaged $18.8 \pm 2.5\%$. Swiss mice inoculated on the same day with the same number of parasitized erythrocytes from Swiss donors had counts which averaged $49.8 \pm 3.3\%$. The differences between the 2 groups of Swiss were significant ($p < 0.001$). On 8 occasions the C_3H-passed strain was passed through Swiss mice twice serially. On second passage through Swiss the average counts were $28.3 \pm 2.4\%$. In 6 instances the C_3H-passed parasites were passed through Swiss a third time and the parasitemia was $34.8 \pm 3.4\%$. The evidence is that the C_3H-passed parasites developed more slowly in Swiss mice on first passage, and it suggests that on further passage its behavior converts to what would be expected of Swiss-passed parasites. By extrapolation, the C_3H-passed strain would behave like a Swiss-passed strain after 4 to 5 passages in Swiss mice.

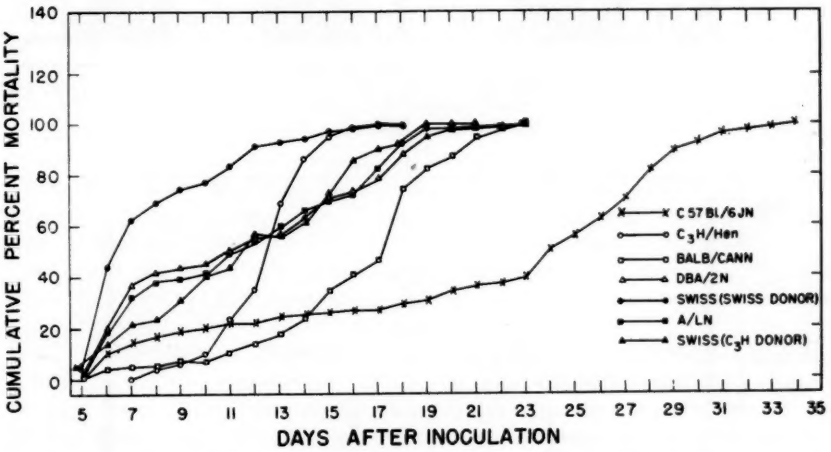

FIGURE 3. Cumulative percent mortality by days after inoculation of *P. berghei* in several strains of mice. Parasite serially passed within each strain.

The survival of all mice except STR is shown in Figures 3 and 4. The survival patterns of A, DBA, and Swiss (C_3H donors) resembled each other. The pattern of all other strains was unique. In every strain but C_3H there was a mode of death around the sixth or seventh day. In all strains there was a principal mode on either the 12th, 18th, or 28th day (C 57 Bl) and suggestion of perhaps minor modes on other days. In every strain but C_3H, the pattern of survival was bimodal with possibility in several of the strains that it was polymodal. The similarities and differences among the survival patterns are perhaps more clearly shown in Figure 4, in which the data are given as cumulative percentage mortality with time.

Table II shows that the percentage of mice surviving day 10 and the day by which 50% of the mice were dead was similar in A, DBA, and Swiss (C_3H donor). About 60% of the mice survived the first peak of death, whereas in Swiss (Swiss-donor) about half as many survived this period of the infection. About 80% of C 57 Bl and 90 or more percent of C_3H and BALB survived the first 10 days. In terms of 50% mortality A, DBA, C_3H and Swiss (C_3H donor) were similar. In

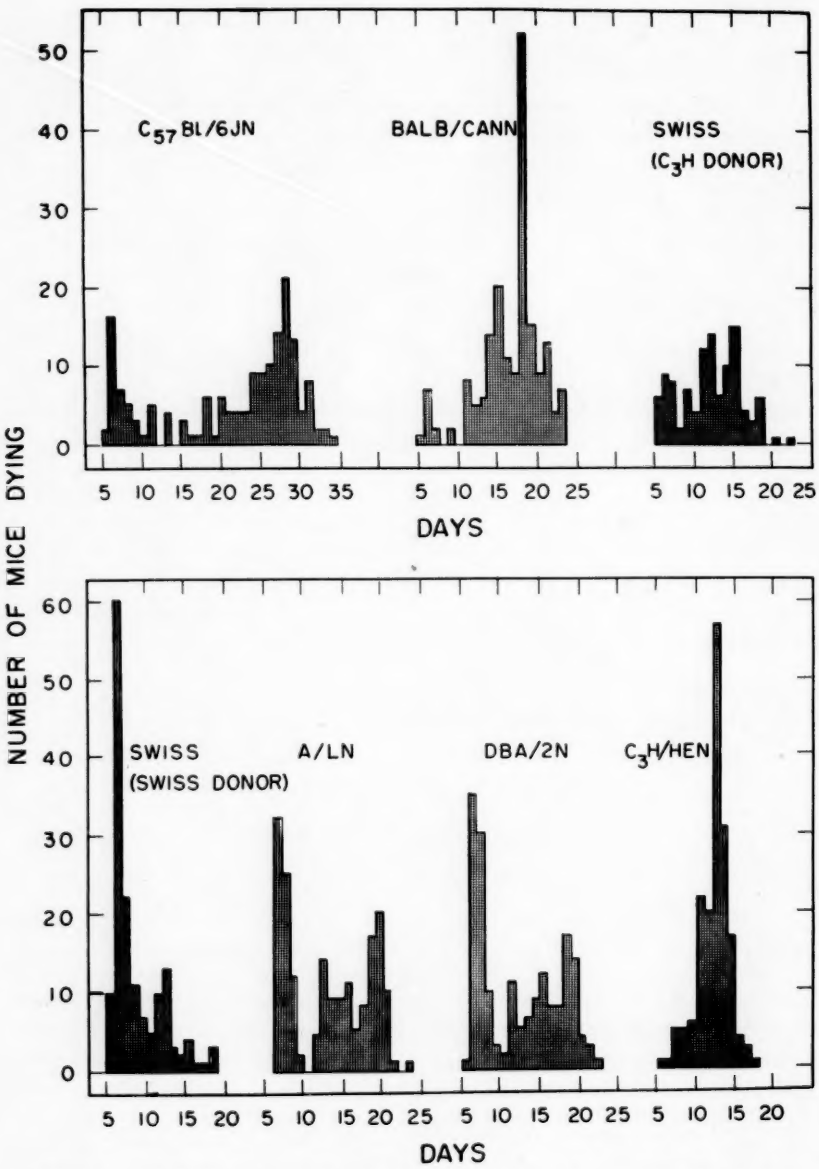

FIGURE 4. Mortality by days after inoculation of *P. berghei* in several strains of mice. Parasite passed serially within each strain.

terms of mean survival, useful only for rough comparisons, the last strains of mice formed a comparable group. It is obvious that mean survvial and 50% mortality data mask the very large difference in survival patterns between C_3H and the group of mice including A, DBA, and Swiss (C_3H donor). It is also apparent in

TABLE II — *Mortality in mice of six strains after inoculation with* P. berghei, *passed within each strain*

Mouse Strain	% dead by day 10	50% dead by day	Mean survival (days)
Swiss (Swiss donor)	77	7	7.7
Swiss (C_3H donor)	33	12	11.7
A/LN	39	12	12.5
DBA/2N	45	11	11.8
C_3H/Hen	10	12	12.7
BALB/CANN	7	17	16.5
C 57 B1/6JN	19	24	20.0

spite of the fact that more BALB and C_3H mice survived the first peak of death than did C 57 Bl, the latter mice on an average outlived the first two. No single, simple factor can be used to describe mortality in mice.

DISCUSSION

These experiments were designed to determine whether, on passage through a particular strain of mice, *P. berghei* "adapted" to that strain. Specifically the criterion for adaptation was an increased rate of invasion of mature erythrocytes as measured at the end of the first week. In none of the 6 strains of mice was there any evidence of adaptation after 21 consecutive weekly passages. One can conclude that the relatively high parasitemia observed in Swiss mice is a characteristic of that strain of mouse. It was coincidental, and irrelevant with certain limitations, that *P. berghei* had been passaged through Swiss mice and that these mice had consistently higher infections than other strains studied.

Not only was there no evidence of increased parasitemia on passage of the parasite through inbred strains of mice, but in C_3H mice there was a dramatic reduction in parasitemia as the organism was passed in this strain. A somewhat similar, but less dramatic, drop in infection rate was seen in STR mice, but this is not as well documented. No such decrease in rate of invasion of mature erythrocytes occurred in any of the other strains of mice studied.

It is perhaps significant that the changes in C_3H mice from high to low parasitemia occurred gradually over 4 or 5 passages. It is also significant that the C_3H-passed parasite produced a lower infection when first passed in Swiss mice than did Swiss-passed parasites. On continued passage in Swiss mice the C_3H-passed parasite appeared to reacquire the characteristics of the Swiss-passed parasites.

There is no explanation for the observed loss of virulence of the parasite in C_3H mice which is supported by evidence. It is possible that, from an originally highly heterogeneous population, passage in C_3H mice selected variants which had lost their capacity to multiply in the mature erythrocyte. Alternatively there may be in Swiss mice a substance which promotes the invasion of mature erythrocytes, this substance being absent in C_3H mice. The nature of the substance would have to be such that it required 4 or 5 passages in C_3H mice to get rid of it and 2 or 3 passages in Swiss mice to reacquire it. Whatever the eventual explanation of the loss of virulence, using the term loosely, the point remains that the characteristics of the parasite were altered by passage through strains of one species of host, permanently within that strain and at least temporarily when transferred

back to another strain. Alterations in biological characteristics are rather the rule when the parasite is transferred from species to species of host, but this does not allow nearly the scope for experimentation as changes produced by strains of host within one species.

If the genetics of the host can alter the virulence of the parasite, this offers some intriguing possibilities for laboratory studies in epidemiology. One could set up mixed populations and determine after an arbitrary number of mouse generations and a number of parasite passages what the mouse population would be like in respect to resistance to the infection and what the biological characteristic of the parasite would be like in respect to virulence. Before this could be done, several simple questions require answering; for example, what would be the fate of the parasite, originating from Swiss mice or alternatively from C_3H mice, when it is passed through various hybrid mice of which C_3H was one parent? This would answer such questions as to whether the characteristic of C_3H mice which suppresses the infection in mature erythrocytes is inherited as a dominant or recessive. Is the inheritance mechanism simple or complex?

Turning to the data on mortality, the Swiss mice in the present series compare with the Swiss mice in the previous series (Greenberg and Kendrick, 1956). In the previous series 77% of the Swiss mice were dead by day 10, and 50% by day 6 (Table II). The differences between C 57 Bl inoculated from Swiss donors and from C 57 Bl donors were small. In the former series 13% were dead by day 10 and 50% by day 20. In the other 4 strains the differences between the 2 series were large and apparently real. In the Swiss-donor series between 70 and 80% of the A, DBA, and C_3H mice were dead by day 10, and 50% were dead by the 6th or 7th day; their mortality curves were almost exactly replicates of that observed in Swiss mice. When inoculated with parasites carried within the strain, there was a dramatic decrease in the percentage of mice which died in the early wave of death. The same was true of BALB mice. Only in C_3H mice could the decline in early mortality be ascribed to a decrease in parasitemia in the first week of the infection. In the other strains the percentage of mature erythrocytes invaded during the first week was essentially the same whether one inoculated Swiss-passed parasites or within-strain parasites. This must mean that a large percentage of the early mortality observed in A, DBA, and BALB mice, when Swiss donors were used, was associated with having received their infection from a foreign donor or perhaps more specifically from a Swiss donor. It will be noted that the mortality pattern of A and DBA mice in the current series still resembled each other, but that in C_3H mice very few died in the early wave of deaths. It seems reasonable that the additional decrease in early mortality in C_3H mice be attributed to the reduction in parasitemia of mature erythrocytes. Whether this reduction in parasitemia explains the marked diminution in early deaths in Swiss mice inoculated from C_3H donors is not entirely clear. The survival pattern of these mice is remarkably like that of A and DBA mice and this may signify that even in Swiss mice Swiss-passed parasites are more lethal apart from their ability to invade mature erythrocytes.

There are some worthwhile comparisons in survival among the strains used. For one, even though a larger percent of C_3H mice survived the first wave of deaths than did DBA and A, the overall survival among these strains was the same.

This was because the major wave of deaths among C_3H mice occurred about the 12th day, while in DBA and A mice it occurred about a week later. This is further illustrated by comparing C_3H and BALB mice. In both of these strains about the same number of mice survived the 10th day, yet on the average, BALB mice outsurvived C_3H mice by several days. Again the major wave of deaths in BALB mice occurred about a week later than in C_3H, or about the same time it occurred in A and DBA mice. On the other hand, about twice as many BALB mice as C 57 Bl mice survived the early wave of death, yet on the average C 57 Bl mice outlived BALB mice. Again this can be explained on the basis of when the major wave of deaths occurred. In C 57 Bl this occurred about the 28th day.

What began as a simple observation that some strains of mice survive longer when infected with *P. berghei* than others (Greenberg, Nadel, and Coatney, 1954) has now become a complex problem involving several related but apparently independently inherited characters: (1) The percentage of mice which survive the first wave of death, which is related in some manner to (2) the percentage of mature erythrocytes which are infected by the end of the first week. This in turn is a function of the strain of mouse and the source of parasite. (3) The degree of parasitemia depends in some instances on whether the parasite is carried within the strain of mouse or not. (4) Survival with malaria is a function of the time of occurrence of the second major wave of death. Strains of mice carry genes which influence each of these variables and from the little that is so far known about the mechanism of inheritance of these characteristics, each is controlled by a complex of genes rather than by any simple 1 or 2-gene system.

SUMMARY

Plasmodium berghei was transferred serially once weekly for 21 weeks through each of 7 different strains of mice. The percentage of mature erythrocytes infected at the end of the first week varied from 1 to 2% in C_3H and STR mice to 47% in Swiss mice. The results were compared with previous experience where Swiss donors had been used as a source of parasites to infect mice of the same strains. In 3 strains (C 57 Bl, BALB, and DBA) it made little apparent difference whether Swiss or within-strain donors were used. In one strain (A) the counts were about 10% lower when within-strain donors were used. In one (C_3H) and possibly another (STR) there was a gradual and significant decrease in parasitemia as the parasite was transferred within the strain of mouse. The slower multiplication rate of C_3H-passed parasites was apparent through at least 1 transfer into Swiss mice.

In general, mice infected with within-strain passed parasites lived significantly longer than those infected from Swiss donors. This was true regardless of whether there was a reduction in the infection of mature erythrocytes.

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BLOOD PARASITISM IN THE ENGLISH SPARROW, WITH CERTAIN BIOLOGICAL IMPLICATIONS

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Since the English sparrow (or "house sparrow"), *Passer domesticus domesticus,* is one of the commonest of birds, both here and in many other parts of the world, it might seem that everything concerning its parasites would have been discovered long since. To a certain extent this is indeed the case, and yet study of the literature shows many gaps, and also suggests that little consideration has been given to the possible broader biological significance of certain facts, such as seasonal and geographic variations in parasite incidence, differences in incidence among host species of parasitism by certain species or genera, etc.

Our interest in the parasites of the English sparrow (not really a sparrow at all, but a member of the Old World family "Ploceidae," or weaver birds) began a number of years ago, and we have been trapping these birds, except for a period of World War II, since 1935. Most of the birds used in the present study, however, were captured during the past 5 years. Many were taken on the University campus and the rest in the little town of Fayetteville, about 9 miles East of Syracuse.

The method of study has involved the use of Romanowski-stained blood films, the inoculation of other birds (either canaries or other English sparrows) with blood from suspected cases of malarial infection, the examination microscopically of tissue preparations from sparrows dying naturally or killed, and the inoculation of white mice with brain suspensions from such sparrows to reveal possible *Toxoplasma* infections. When sparrows were used as test animals for the discovery of what might be called "occult" malaria in other sparrows, the birds were simply divided at random into pairs, after failure to find evidence of malaria in blood films, and each was then inoculated with 0.25 ml of blood from the other, usually intravenously. Canaries were used for subinoculation from cases already shown to be positive, in order to secure laboratory infections for further study. Mice inoculated with brain suspensions were observed for 2 weeks for indications of toxoplasmosis, and then killed and subinoculations of suspensions from them were made into a second series of mice, before regarding negative findings as confirmed.

A summary of the results of this study and of a number of previous ones appears in Table I. The total number of sparrows examined by us (703), included 303 adults and 400 immatures. These birds have been trapped in every season of the year, although of course no juveniles were caught in the winter or early spring. Although the sparrows studied in different seasons and different years have not been equal in numbers, it is believed that the spread in time has been sufficient to give a reasonably balanced picture, free from such distortions as those which might result from variation in parasite incidence, due to seasonal factors, differences in age distribution, and the like.

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TABLE I — *Summary of blood parasites reported for English Sparrow*

Authority	Area *U. S.*	Examined	Totals Negative	Malarious	Species of *Plasmodium*								Other Parasites					
					catb.	*catb-rel.*	*circ.*	*elong.*	*hex.*	*nuc.*	*rel.*	*rouxi.*	*unid.*	*Atox.*	*Hg.*	*Hp.*	*Leu.*	*Mf.*	*Tryp.*
Grundman *et al.* '52	Utah	209	179	30	yes						yes								
Hart, '49	S. C.	100s	97	2		2	1?	1			3			8		1	2?		
		223w; sp	205	10		4													
Herman, '38	Mass.	41	11											30au					
Herman, *et al.** '54	Cal.	2880*	2638	242							234		8						
Huff, '39	Ill.	125	110	13	5			2			1		5						2
Hunninen & Young, '50	S. C.	225	193	30		2					28		1		1?		1		
Jordan, '43	Geo.	489	418	70	14			3			42		5						1
Love *et al.* '53	Geo.	250	207	42		35	1	5					4			1			
Manwell, '56	N. Y.	703	618	36	10	12	2	2			10		1	41	7			1	
Manwell & Herman* '35	N. Y.	245	238	7							7								
Micks, '49	Md.	516	458	58	5			4			31		18						
Opie, '98	Md.	80	74	5		5										1			
Thompson, '43	Geo.	41	38	3							2		1						
Wetmore, '41	D. C.	9	8											1					
Wood & Herman, '43	Cal.	41	38	2									2		1?				
	Elsewhere																		
Abbass, '55	Iraq	108	96	11							11					1			
Cerny, '33	Cz. Sl.	50	40	4							4					3	1		
Haiba*, '48	Egypt	67			yes			yes		yes	yes	yes							
Lawrence, '46	Aust.	91	26	65		60							5	27					
Singh, *et al.* '51	India	869	575	256							256					49			

* Only malaria looked for

† Two subspecies of sparrow: *Passer domesticus niloticus* (65) and *P. d. chephreni* (2).

s - summer
w - winter
sp - spring
au - findings based on autopsy examination

Abbreviations: *catb. — cathemerium, catb-rel. — cathemerium relictum, circ. — circumflexum, elong. — elongatum, hex. — hexamerium, nuc. — nucleophilum, rel. — relictum, rouxi. — rouxi, unid. — unidentified, Atox. — Atoxoplasma, Hg. — Haemogregarina, Hp. — Haemoproteus, Leu. — Leucocytozoon, Mf. — Microfilariae, Tryp. — Trypanosoma*

RESULTS

Species of malaria found included *Plasmodium cathemerium* and *P. relictum* (these 2 being the most common), and *P. circumflexum* and *P. elongatum*. Mixed infections are doubtless frequent but blood films seldom reveal them, and large scale inoculation into clean birds would have been required to reveal such additional species of *Plasmodium* as may have been present.

Malaria in the acute stage was seen only in immature birds, and such infections were almost always relatively mild, only a few parasites being seen in the course of a standard 5-minute examination. A single moderately severe case, judged such because the level of parasitemia was only about 10%, was seen. Since few reticulocytes were present and there was no evident anemia, it was obviously doing the bird little harm.

Inasmuch as the method of testing for hidden malarial infection by subinoculation between members of random pairs resulted in the discovery of 7 positives among 22 birds so treated, one must conclude that malarial infection in English sparrows is much more frequent than the 3.7% of positive blood films would make it seem to be (Table II). For purposes of comparison, figures for song and chipping sparrows, trapped in the same neighborhood, are included.

TABLE II. *Blood parasites of three species of sparrows.**

Host Species	Total Cases	Neg.	Malaria	Haem.	Leuco.	Atox.	Tryp.	Hg.	Mf.
English sparrow	703	592	26 (3.7%)			40		7	1
Song Sparrow	591	440	112 (18.9%)	18	19	1	2	1	12
Chipping sparrow	155	134	8 (5.1%)	9	3	2		1	

* Diagnosis based on blood smears alone. If the results of subinoculation into clean birds were included, the figures for the incidence of malaria would be higher.

Abbreviations: Neg.—negative; Haem.—*Haemoproteus*; Leuco.—*Leucocytozoon*; Atox.—*Atoxoplasma*; Tryp.—*Trypanosoma*; Hg.—*Haemogregarina* (or other Haemogregarines); Mf.—*Microfilaria*.

The reason for the disproportionately low percentage of positive blood films in the English sparrow seems most likely to be a naturally high resistance to the effects of parasitism, as distinct from the parasite itself—just as the negro appears to be more resistant to *vivax* malaria than does the white man. Thus, even in young birds, the parasitemia seems usually to be relatively low and the duration of patent infection short, with few relapses. Otherwise the number of observed malarial infections would almost certainly have been greater.

Absent were cases of malaria due to *Plasmodium hexamerium, P. nucleophilum, P. vaughani,* and *P. rouxi. Plasmodium rouxi* is only known with certainty to occur in eastern Asia, where it seems to be rather common among the native subspecies of English sparrows, and *P. nucleophilum* has also been recently reported from them, although this needs confirmation. *Plasmodium vaughani* is probably world-wide in distribution, but has never been found in English sparrows. Indeed it seems to be common only in robins and some of their close relatives. *Plasmodium hexamerium,* although common in the song sparrow and some of the other Fringillidae (and occuring rather widely in birds of other families also), has apparently never been found in the English sparrow.

Absent, too, were the malaria-related genera *Leucocytozoon* and *Haemoproteus. Haemoproteus* has been reported from English sparrows in Europe (Prowazek, 1920), and recently from India (Singh *et al,* 1951), but elsewhere it seems to be

very rare in this host. It is also entirely possible that some of the reports of its occasional presence in other parts of the world may have been due to confusion with the gametocytes of *Plasmodium circumflexum,* or even of *Plasmodium elongatum.*

As for *Leucocytozoon,* this parasite has been reported so rarely from the English sparrow that one may properly wonder whether it really occurs at all in this host. The parasite *Atoxoplasma,* often called "avian *Toxoplasma,*" but quite distinct and recently renamed *Atoxoplasma* by Garnham, (1950), may easily be mistaken for a young *Leucocytozoon.*

Atoxoplasma is perhaps actually the most common blood parasite of the English sparrow, and it is seen especially often in young birds. I have found it recently in at least 1 of every 5 young sparrows examined. In lung smears it is often abundant.

In this connection, the question may be asked whether *Atoxoplasma,* or "avian *Toxoplasma*" may not in reality be a name applied to the stages of some avian coccidian occuring in the blood, as suggested by Coulston (1942). This is indeed possible, but to the author it seems unlikely. These parasites do not have the morphology typical of coccidia, and while coccidiosis in sparrows is very common, much more than an association of blood *Toxoplasma* and intestinal coccidial infection is required to establish their essential identity. The sparrows examined in the present study were not studied for coccidiosis, however.

Only 1 case of microfilarial infection was revealed, but these parasites are much more likely to be seen *post mortem* in heart and blood smears, and few such examinations were made. Nor were any cases of trypanosomiasis observed, a rather surprising fact when the number of birds is considered, and particularly the relatively high proportion of young ones. However blood films have been found much less likely to reveal such infection in birds than blood- or marrow-inoculated cultures (Diamond and Herman, 1954).

Ectoparasites, other than the common bird mite, thought to be *Dermanyssus gallinae,* were also not seen. Even this mite was much less frequently found on English sparrows than on song sparrows and other small birds.

No cases of toxoplasmosis were discovered, although more than 250 English sparrows were killed and brain suspensions from them inoculated into white mice. Nor, apparently, has anyone else yet found it in this avian host. Nevertheless, we are still looking for it, both because this sparrow is often seen in haunts frequented by the pigeon, which has long been known to be a host of *Toxoplasma,* and because of the house sparrow's association with man, also often a host, and its more or less omnivorous diet.

The English sparrow is susceptible to infection by intraperitoneal inoculation with *Toxoplasma,* and may exhibit either a rapidly fatal or a chronic form of the disease. Of 40 birds so inoculated, none has yet been found either refractory to the infection, or to have recovered naturally. The longest survival time so far observed has been 54 days. However, these birds were killed for tissue study, and it is impossible to know how much longer they might have lived. Although the birds sacrificed were apparently healthy, the parasites in their brains were infective to mice. For most of these experiments the RH strain, of human origin, was used, but the results of experimental infection with *Toxoplasma* derived from the crow have been similar.

DISCUSSION

These findings raise certain questions: Is the relative rarity of some blood parasites in the English sparrow due to a higher resistance or perhaps to ecological factors? Would different methods of search possibly have revealed a significantly higher incidence of parasitism? One may also wonder whether differences in parasite incidence shown in different studies may not be connected with geography. There are numerous problems in relation to life histories. Nothing, for example, is known about *Atoxoplasma*, other than the appearance of the parasites, and their apparent preference for lymphocytes as host cells.

The vectors of the avian malaria parasites are also incompletely known. Perhaps some of the peculiarities in the incidence of the various avian malarias in the English sparrow may be correlated with the biting habits of their transmitters. But it seems hardly likely that the absence of *hexamerium* malaria can be so accounted for, since experimental inoculation of the English sparrow with this parasite usually fails to produce infection. Nor can the freedom of locally occurring English sparrows be due to the absence of the vector, for young song sparrows have been frequently found infected.

The rarity of *Leucocytozoon* and *Haemoproteus* in the English sparrow is remarkable, when one considers how common both these genera are in many species of birds—the incidence sometimes approaching 100%, with mixed infections extremely frequent. Published reports, as shown in Table I, indicate that perhaps only in India is *Haemoproteus* common in English sparrows (Singh *et al*, 1951). However it was early found in this host in Europe (Prowazek, 1920, quoting Galli-Valerio, 1902), although nothing is said about how commonly it was found. Since neither *Plasmodium circumflexum* nor *P. elongatum*, both of which produce elongate gametocytes considerably resembling those of *Haemoproteus*, were then known, one cannot be sure of the correctness of such early reports.

Any freedom which the English sparrow may enjoy from infection by these parasites must be a specific characteristic, for other species of Ploceidae have often been found harboring both *Haemoproteus* and *Leucocytozoon*. A more likely reason may be the absence of suitable vectors. Hippoboscids, the usual vectors of *Haemoproteus*, are often quite host-specific. *Simulium*, species of which transmit *Leucocytozoon*, are however generally less so.

The relative rarity of *Haemoproteus* and *Leucocytozoon* in North American English sparrows (or, it may be, their complete absence) in an environment in which such infection is so common among other birds, also seems to be evidence of the usually assumed, but never proved, strict host-parasite specificity in these 2 genera.

It also suggests that, as so often happens, some of the parasites of the English sparrow may have been left behind as it spread to, or was introduced into new regions. Locally occurring species of *Haemoproteus* and *Leucocytozoon* seem not to have been adaptable enough to extend their host range to the new arrival.

There are reports in the literature, including that of Zasukhin, *et al* (1949) of toxoplasmosis in the English sparrow in Russia, but this, like most of the earlier reports, appears to have been based only on the examination of blood films. The author himself was responsible for one such report in 1941, although it was made with some reservations, since attempts to transmit the infection to canaries (which are highly susceptible to toxoplasmosis) proved unsuccessful. Until a strain of *Toxoplasma* is actually isolated from English sparrows, and established in laboratory animals such as the white mouse, all such reports must be viewed with suspicion.

SUMMARY AND CONCLUSIONS

The blood parasite picture of the English sparrow appears to differ significantly from that of numerous other species of birds sharing a more or less similar environment. This sparrow seems to have a relatively higher resistance to some species of avian malaria, so that such infections appear rarely or not at all. *Leucocytozoon,* although extremely frequent in most avian species, is either very rare or perhaps does not occur in the house sparrow. *Haemoproteus* also probably does not occur in this host in North America, and is evidently rare except in India. *Atoxoplasma,* formerly usually called "avian *Toxoplasma*", on the other hand, though widely distributed in birds, seems especially common in this species of sparrow. *Toxoplasma gondii* has not yet been found, but the English sparrow is easily susceptible to experimental infection which often becomes chronic, just as it does in the pigeon. For this reason, and because of the close association of the English sparrow with man and species such as the pigeon in which naturally acquired toxoplasmosis is common, it should be regarded as at least a potential host.

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RESEARCH NOTE

OBSERVATIONS ON THE LIFE CYCLE OF *THELAZIA CALIFORNIENSIS* PRICE, 1930

The nematode *Thelazia californiensis* Price, 1930, has been found in the eyes of mammals in California and in some adjacent areas of Oregon and Nevada. Known definitive hosts are the dog, deer, cat, horse, sheep, coyote, bear, and man.

Preliminary results on studies of the intermediate host involved in the life cycle of *T. californiensis* are reported here. Both experimentally and naturally infected vectors were used in the study. Sheathed embryos of the worm were fed to several species of laboratory-reared flies which were examined at intervals by crushing under a cover slip. Developmental forms of the worm were found in *Fannia canicularis* (Diptera: Muscidae). Similar developmental forms of a nematode were found in specimens of *F. benjamini* collected near San Bernardino, California, an area endemic for the eye worm. It is believed that these are the results of natural infections of the parasite.

This note constitutes the first report of intermediate hosts for *T. californiensis* or for any species of this genus in the United States. This is in agreement with work done in Russia on 3 species of *Thelazia* for which species of Muscidae were found to be the intermediate hosts.—HOWARD S. BURNETT, WARREN E. PARMELEE, ROBERT D. LEE, EDWARD D. WAGNER, *School of Tropical and Preventive Medicine, College of Medical Evangelists, Loma Linda, California.* (This is part of a study being done on U. S. Public Health Service Grant E-951.)

OBSERVATIONS ON THE MODE OF INFECTION OF THE HUMP OF CATTLE BY *STEPHENOFILARIA ASSAMENSIS* IN EAST PAKISTAN

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Although hump sore of cattle has long been known, the information available on this subject is rather meager. The genus *Stephenofilaria* was first established by Ihle and Ihle-Landenberg (1933) and up to this time only 4 species have been created. *Stephanofilaria assamensis* is the name given by D. Pande (1936) in Assam where he collected some filarias from hump sore of cattle.

The following data were collected in a survey of cattle in East Pakistan. It was also observed that the percentage of infection with hump sore is greater in the preserves, corrals, and dairy farms than in villages. Imported Sindhi and Hariana cattle are more susceptible than the local breed.

Localities visited Districts	No. of villages and cattle markets visited	No. of animals examined	No. of affected cattle	Percentage of infection
Comilla, Dacca, Mymensingh, Sylhet, Chittagong, Khulna, Noakhali, Barisal, Hill Chittagong, Rajshahi and Rangpur.	250 villages. 25 cattle markets.	25,000	625	Average 25%

The disease is seldom observed in young cattle under the age of 7 years. Accidental infection was seen in only 1 bull calf. Male cattle are more prone to infection than female. Strong and healthy cattle are equally as susceptible as weak animals.

Severity of infection in different seasons varies slightly but cases were found to be aggravated with the onset of monsoon rains when flies are abundant and the farmers in East Pakistan are compelled to keep their animals in the cow shed for a long period due to flood. Although the parasite has a special affinity for the hump of cattle, the infection of the skin on the other parts of the body is not totally absent.

EXPERIMENTAL

An experiment on the transmission of infection from animals with hump sore to healthy cattle was done at Comilla. Two bullocks with positive hump sore and 1 bullock and 1 cow without hump sore were kept together in a fly-proof shed from January 26 to February 16, 1956. About 100 flies were allowed to enter the shed with the cattle. Next day 900 more flies were caught from the field and sweetmeat shop and these flies were introduced into the shed. The cattle and the flies were not allowed to escape from the shed. Flies were not given any extra food in the shed but had to procure their food from the cattle. Day-to-day observation of the healthy cattle revealed no sore on their humps. During that period 100 flies were dissected. Of these, 7 were found with filarial larvae on their body surface but no such larvae could be detected inside the probosis or in the gut. Flies were put in water in a watch glass before dissection and the larvae were seen moving on the body of the flies. On agitation the parasites were detached from the flies

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and found moving free in water in the glass. These were identified as larvae of *Stephenofilaria assamensis*. No mosquito was allowed to remain in the shed.

On Feb. 20, 1956, scraping sores were artificially created on either side of the hump of the same bullocks and only the cow was kept as a control. The cattle were kept in the same fly-proof shed and some more flies were introduced. Filarial larvae were recovered from scrapings of the experimental bullocks on Feb. 24, 28, March 2, 7, and later. Scraping was done on every 4th day. The animals were kept in the shed for another 21 days and during this period 200 flies were dissected. Of these, 35 flies were found with larvae on their body surface. The cow kept as control developed no hump sore though she lived 42 days with 2 hump sore cattle.

DESCRIPTION OF THE PARASITE

Genus Stephenofilaria Ihle and Ihle-Landenberg, 1933. The species *S. assamensis* Pande, 1936 was discovered in Assam from hump sore of cattle. This nematode is viviparous. *Host*:—Cattle; location, hump sore; locality, East Pakistan. The female measures 9 to 11.5 mm long and 105 microns wide; male measures 3.5 mm long and 105 microns wide; esophagus 84 microns long; spicules unequal, one 133 microns long and the shorter one 42 microns long. Body striated transversely. Vulva opens 420 microns posterior to the oral opening. In the female, uterus occupies almost entire body of the worm. There are 3 papillae a little posterior to the oral opening. Mouth is a simple opening with very fine lip-like processes. The proportion of male worms to female is 1 : 10 as found in the scrapings.

DISCUSSION

Hump sore is a chronic skin disease and the lesion is generally confined to the hump of adult cattle. The infection in other parts of the body than the hump is very rare. The filaria is mostly confined to the skin very superficially. There is only one instance in which it was found in blood taken from the ear vein. The animal shows neither rise of temperature nor any systematic disturbance. Therefore the presence of *S. assamensis* in the blood circulation can safely be said accidental and non-pathogenic.

In the reports of past workers none has mentioned *Stephenofilaria assamensis* as viviparous. The female *S. assamensis* were found laying larvae in the Petri dishes on microscopic examination immediately after collection from hump sores. Eggs inside the uterus are arranged in the grade of maturity starting from the opening of the vulva; i.e. the larvae are set in a queue for delivery, according to maturity. The infection is direct and the transmission is just mechanical, through flies, mosquitoes, other arthropods, and birds which feed on the hump sore.

To transmit the larvae from the diseased cattle to cattle without hump sore a previous wound or breaking of the skin is necessary. A lymph-secreting wound is generally created by cattle themselves by rubibng the side of the hump against some rough surface, generally against the trunk of trees. In such a wound, flies find ample food and gradually transmit the larvae from neighbouring infected cattle. When a few larvae are transmitted the irritation increases and rubbing of the parts aggravated. The transmitted larvae become mature and start laying larvae and within the course of few months a fully developed hump sore appears.

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OXYSPIRURA (*YORKEISPIRURA*) *PUSILLAE* N. SP. (NEMATODA: THELAZIIDAE) FROM THE ORBITAL CAVITY OF THE BROWN-HEADED NUTHATCH, *SITTA PUSILLA PUSILLA* LATHAM, 1790

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A number of specimens of a nematode were recovered from the orbital cavity of a brown-headed nuthatch, which was captured near Emory Field Station, Newton, Georgia, by Mr. R. A. Norris and sent to one of the authors (EEW) for identification. A study of these specimens showed that they belong to the genus *Oxyspirura,* subgenus *Yorkiespirura,* and represent a new species.

Oxyspirura (*Yorkeispirura*) *pusillae* n. sp.

Description. Oral opening more or less hexagonal in outline. Lips rudimentary; pseudolabia absent. Papillae of external circle consisting of 4 well-developed papillae and 4 reduced papillae; papillae of internal circle much reduced in size and 6 in number. Buccal capsule divided by a distinct constriction into 2 well-developed, cylindrical parts (Fig. 4); anterior portion wider and shallower than posterior portion No annulations or rugosities present in buccal capsule.

Male (Holotype). (All measurements in millimeters) 6.4 long and approximately 0.232 wide near middle of body. Buccal capsule 0.045 long; anterior portion approximately 0.025 wide and 0.02 deep; posterior part 0.0176 wide at its widest point and about 0.025 deep. Esophagus 1.1 long, club-shaped; 0.043 wide at anterior end and 0.098 wide at posterior end. Nerve ring 0.23 and excretory pore 0.332 from anterior end (Fig. 1). Spicules dissimilar and unequal, alated, with alae extending beyond tips in a fanlike shape (Fig. 2). Longer spicule 2.4 long, relatively slender; shorter spicule 0.211 long, stout. Gubernaculum 0.084 long, narrow (Fig. 2). Caudal alae absent. Caudal papillae 12 in number; 4 pairs situated preanally and slightly more laterally than others, 2 pairs posterior to anal opening, situated close to median ventral line and not always distinctly visible when viewed laterally (Fig. 3). Phasmids near tip of tail.

Female (Allotype). 9.3 long and approximately 0.297 wide at widest point. Length and width of buccal capsule and esophagus similar to male as regards size relations, but proportionately larger. Vulva postequatorial, 0.64 from posterior end of body, and with a cuticular thickening around its opening (Fig. 5). Posterior end of body ending in a small, knob-like structure. Anal opening approximately 0.226 from posterior end of body. Eggs thick-shelled, approximately 0.046 long and 0.027 wide (Fig. 6); more matured ones containing embryos.

Host. Brown-headed nuthatch (*Sitta pusilla pusilla* Latham, 1790).

Location. Under nictitating membrane of eye.

Locality. Emory Field Station, Newton, Georgia.

Type specimens. Holotype (male) and *Allotype* (female) (No. 47482) and *Paratype* (No. 47483) in U. S. National Museum Helminthological Collection.

Skrjabin (1931) proposed the subdivision of the genus *Oxyspirura* Drasche, 1887, into 3 subgenera, based on the structure of the mouth capsule and character of the spicules. Since the publication in which Skrjabin's paper appeared is not readily available to many workers in the field of nematode morphology, it seems desirable to include here his original description of these 3 subgenera. He defined these as follows:

"1. *Oxyspirura* n. subg. Die Mundkapsel ist ungeteilt, die Spicula von verschiedener Grösse, aber fast gleicher Struktur.

Typus O. (*O.*) *cephaloptera* (Molin 1860). . . .

"2. *Yorkeispirura* n. subg. Die Mundkapsel ist durch eine Einschnürung in zwei Teile geteilt, die Spicula sind von verschiedener Grösse und Struktur (das eine fedenartig dünn, das andere verdickt).

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Typus: *O.* (*Y.*) *mansoni* (Cobbold 1879). . . .

"3. *Cramispirura* n. subg. Mundkapsel ungeteilt, die Spicula fast gleich gross und gleich gebaut.

Typus: *O.* (*C.*) *popowi* n. sp."

Cram (1937) reviewed the genus *Oxyspirura* and included keys to subgenera as proposed by Skrjabin and to the species of each subgenus. As a result of this study, several species which had been designated as only *Oxyspirura* species or placed only in the genus were placed subgenerically as well. Under the subgenus *Yorkiespirura,* she included only 3 species, namely, *O.* (*Y.*) *tanasijtchuki* (Skrjabin, 1916), *O.* (*Y.*) *tsingchengensis* (Hsü, 1933), and *O.* (*Y.*) *mansoni* (Cobbold, 1879). Two years previously, in 1935, Baer redescribed *Spiroptera conjunctivialis* Linstow, 1907, which was collected from the orbital cavity of a lemur dying at the Zoological Garden in Berlin, and made the combination *Oxyspirura* (*Yorkeispirura*) *conjunctivialis* (Linstow, 1907). Baer's article apparently was not available to Cram at the time she prepared her treatise on the genus *Oxyspirura* as the above species was not listed in her paper. *Oxyspirura crassa* Caballero, 1938, was included in the subgenus *Yorkeispirura* by Skrjabin, Shikhobalova, and Sobolev in 1949. The original description of *O. crassa* by Caballero (1938) did not include a statement to the effect that the buccal capsule was divided into 2 parts by a constriction nor does the illustration show it to be such. Specimens of *O. crassa* which were identified by Caballero and deposited in the U. S. National Museum Helminthological Collection, Beltsville, Maryland, were re-examined by the authors. They found that the buccal capsule was not divided into 2 parts by a constriction and the spicules were subequal and of similar structure. Therefore, the authors feel they are justified in removing this species from the subgenus *Yorkeispirura* and replacing it in the subgenus *Oxyspirura* where Caballero originally placed it. Another species of the subgenus *Yorkeispirura, Oxyspirura* (*Y.*) *octopapillata,* was described by Caballero in 1942 from the orbital cavity of a cheriway carrion hawk, *Polyborus cheriway.*

The species described herein resembles very closely *O.* (*Y.*) *tsingchengensis* (Hsü, 1933), but differs from it in the presence of a cuticular thickening surround-

TABLE I.—*Comparative measurements of* O. (Y.) pusillae *n. sp. and two similar species*

	O. (*Y.*) *pusillae* n. sp.*	*O.* (*Y.*) *mansoni***	*O.* (*Y.*) *tsingchengensis****
Male			
Length	6.4 – 7.8 mm.	10 –16 mm.	7.77 – 9.1 mm.
Width	0.211– 0.248 mm.	0.2 – 0.35 mm.	0.24 – 0.28 mm.
Stoma	0.035– 0.045 mm.	..	0.036– 0.056 mm.
Esophagus	0.99 – 1.3 mm.	..	0.930– 1.080 mm.
Nerve ring	0.203– 0.23 mm. from anterior end	..	0.185– 0.230 mm.
Excretory pore	0.262– 0.332 mm. from anterior end	0.35 – 0.4 mm.	..
Long spicule	2.2 – 2.4 mm.	3 – 3.5 mm.	2.06 – 2.25 mm.
Short spicule	0.205– 0.22 mm.	0.2 – 0.22 mm.	0.20 – 0.256 mm.
Female			
Length	8 –10.9 mm.	12 –18 mm.	9.37 –11.7 mm.
Width	0.025– 0.03 mm.	0.041– 0.043 mm.	0.300– 0.40 mm.
Stoma	0.038– 0.0435 mm.	..	0.045– 0.057 mm.
Vulva	0.64 – 0.86 mm. from posterior end	1 – 1.4 mm.	0.670– 0.850 mm.
Anal opening	0.226– 0.31 mm. from posterior end	0.4 – 0.53 mm.	0.220– 0.306 mm.
Egg	0.033– 0.05 mm. by 0.026– 0.029 mm.	0.05 – 0.065 mm. by 0.04 – 0.045 mm.	0.040– 0.047 mm. by 0.027– 0.028 mm.

* Measurements based on 3 ♂♂ and 3 ♀♀.
** Measurements as taken from Ransom (1904).
*** Measurements as taken from Hsü (1933).

ing the female genital opening, and the long spicule without undulations. It also resembles very closely *O.* (*Y.*) *mansoni* (Cobbold, 1879), the chicken eyeworm, but differs from it in the shorter spicules and in the presence of a cuticular thickening surrounding the female genital opening. Other significant differences between the 3 species are listed in the table.

The following key will aid in the differentiation of the species of the subgenus *Yorkeispirura:*

1. Stoma (buccal capsule) 0.17 mm deep; long spicule 0.6 mm long
O. (*Y.*) *tanasijtchuki* (Skrjabin, 1916)
Stoma much shallower; long spicule much longer than above 2
2. Caudal papillae numerous, 9 pairs of preanal and 8 pairs of postanal
O. (*Y.*) *octopapillata* Caballero, 1942
Caudal papillae less numerous than above 3
3. Long spicule more than 3 mm long and at least 15 times the length of the short spicule
O. (*Y.*) *mansoni* (Cobbold, 1879)
Long spicule more than 1 mm long and less than 3 mm or not over 12 times the length of the short spicule 4
4. Long spicule not over 2 mm long or about 9 times the length of the short spicule
O. (*Y.*) *conjunctivialis* (Linstow, 1907)
Long spicule over 2 mm long or about 10–11 times the length of the short spicule 5
5. Vulva with prominent cuticular thickening surrounding its opening; long spicule not undulating ..... *O.* (*Y.*) *pusillae* n. sp.
Vulva without prominent cuticular thickening surrounding its opening; long spicule undulating ..... *O.* (*Y.*) *tsingchengensis* (Hsü, 1933)

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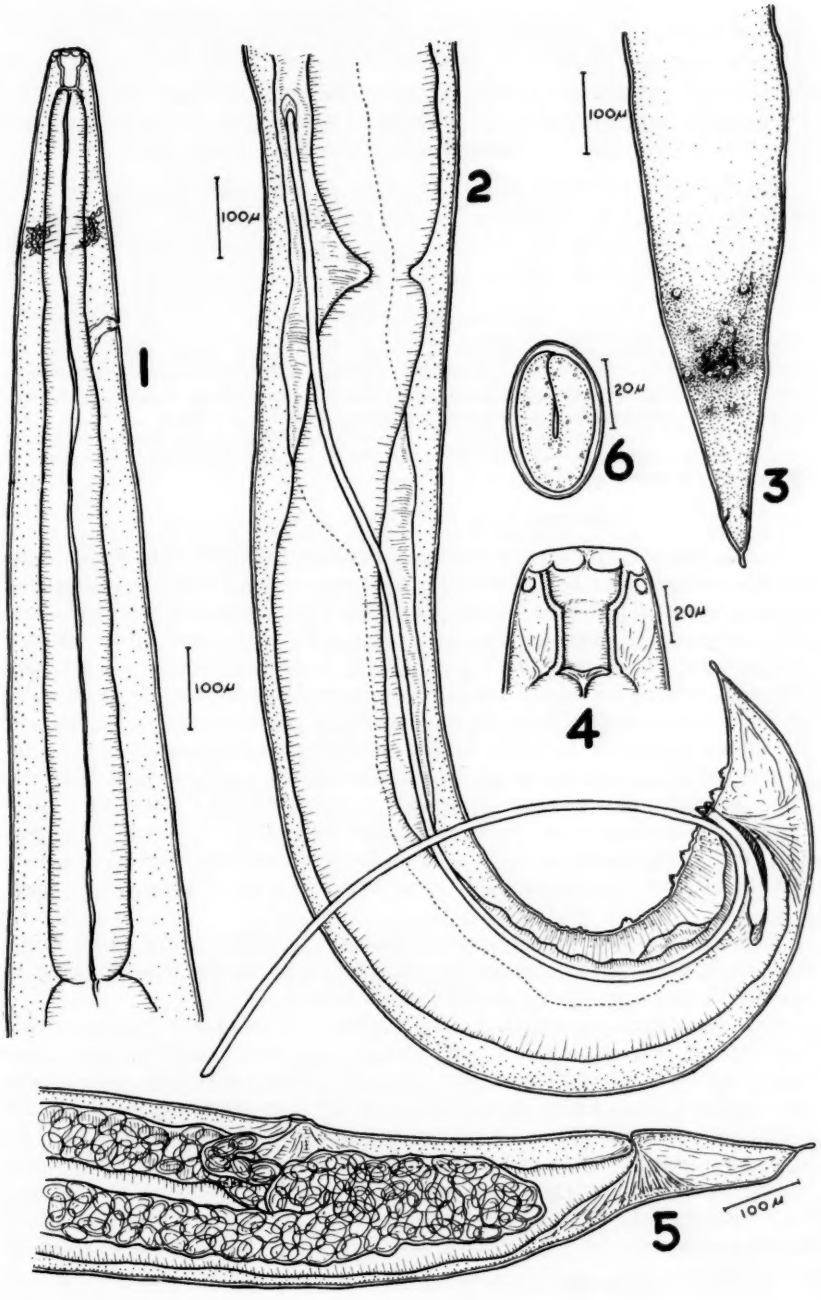
Explanation of Plate

Oxyspirura (*Y.*) *pusillae* n. sp.

Figure 1. Anterior end of male showing buccal capsule, nerve ring, excreting pore and esophagus.
Figure 2. Lateral view of posterior end of male showing spicules and papillae.
Figure 3. Ventral view of posterior end of male showing arrangement of papillae.
Figure 4. Enlarged view of buccal capsule showing division into two parts.
Figure 5. Lateral view of posterior end of female showing vulva and anal opening.
Figure 6. Embryonated egg.

PLATE I



THE EFFECTIVENESS OF CADMIUM OXIDE AGAINST *TRICHINELLA SPIRALIS* IN MICE

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The findings of Burch and Blair (1955) that cadmium oxide is an effective swine ascaricide suggested the possibility that this chemical might exert a demonstrable anthelmintic effect against *T. spiralis* in mice. The present report deals with experiments designed to test this possibility.

MATERIALS AND METHODS

The various experimental procedures were those described in earlier reports from this laboratory (Larsh and Kent, 1949; and others). The test diets (medicated pellets containing 0.015% cadmium oxide, and non-medicated pellets fed to controls) were supplied by Pitman-Moore Co., New Augusta, Indiana, through the courtesy of Dr. G. R. Burch.

Students "t"-test was used to determine the statistical significance of the observed differences in numbers of parasites recovered from the various groups of mice. A probability greater than 0.05 was not considered significant.

EXPERIMENTAL PROCEDURES AND RESULTS

It was necessary first to determine to what extent the test diets would be accepted by our mice, and how effective the consumed amounts would be in maintaining the animals. These determinations were made by preliminary experimentation using the stock diet (Purina Chow) as a standard for comparison. After allowing a few days for adjustment to the change in diet, records were kept of the daily feed consumption over a period of 1 week. These showed that the mice (3 months old) fed the medicated feed (0.015% cadmium oxide) in pellet form, those fed the same feed without medication, and those fed the stock diet consumed, respectively, 2.2 gm, 3.0 gm, and 2.9 gm per mouse per day. Those fed the medicated feed showed no change in weight, whereas those of the other 2 groups showed a slight gain. All of the mice appeared to remain healthy as judged by activity, condition of the coat, eyes, etc. Thus, despite the fact that those fed the medicated feed consumed less feed, the test diets were considered to be satisfactory for the purposes of the present study.

Experiment I. This experiment was designed to determine the effect of cadmium oxide, given by 3 different feeding schedules, on the number of adult *T. spiralis* recovered 7 days after a single infection of 200 larvae.

Forty-eight mice, 3 months old, were selected. On the basis of weight and sex, they were divided equally into 2 main groups, experimentals and controls. Each main group of 24 mice was subdivided into 3 groups of 8 mice each. The experimental mice of Group I were fed the medicated feed from 48 hours prior to infection to necropsy (total of 9 days), those of experimental Group II from the time of infection until necropsy (total of 7 days), and those of Group III from 48 hours after infection until necropsy (total of 5 days). The controls, Groups I, II, III, were fed the non-medicated feed during the same periods that the corresponding experimental groups were fed the medicated feed. At 7 days after infection, all of the

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mice were killed and in each a count was made of the number of adult worms present in the small intestine. These findings are shown in Table I.

The average numbers of worms recovered from the experimental mice of Groups I, II, and III (40.5, 63.1, 80.1) are significantly lower than those from the corresponding control groups (115.1, 102.6, 122.6). It is also of interest to point out that the count for experimental Group I (40.5) is significantly lower than the count for experimental Group III (80.1), while the counts for the 3 control groups are similar.

Experiment II. The design of this experiment was the same as that of Experiment I, except that the period from infection to necropsy was extended from 7 to 9 days after infection. By this arrangement, the experimental mice of Group I received the medicated feed for a total of 11 days, those of Group II for 9 days, and those of Group III for 7 days. The results of the adult counts are shown in Table II.

The average numbers of worms recovered from the experimental mice of Groups I, II, and III (27.1, 46.9, 89.7) are significantly lower than those from the corresponding control groups (154.9, 147.9, 141.2). Also, it should be added that while the counts for the 3 control groups are similar the average count for experimental Group I (27.1) is significantly lower than that for experimental Groups II (46.9) and III (89.7), and that the Group II count is significantly lower than the Group III count.

Experiment III. The design of this experiment was the same as that for Experiment II, except that the period from infection to necropsy was extended from 9 days to 30 days after infection when counts were made in each mouse of the number of larvae isolated from the musculature. After discontinuing the test diets on the 9th day after infection, both the experimentals and controls were fed the stock diet (Purina Chow) until necropsy. Thus, the test diets were fed for the same period of time as in Experiment II (i.e., for 11, 9, and 7 days, respectively, for Groups I, II, and III). The estimated average number of larvae recovered from each group of mice is shown in Table III.

The average numbers of larvae recovered from the experimental mice of Groups I, II, III (3,996; 7,610; 13,408) are considerably lower than those from the corresponding control groups (33,475; 39,065; 33,282). The count for experimental Group I (3,996) is significantly lower than that for experimental Group III (13,408), while the counts for the 3 control groups are similar.

Experiment IV. The previous experiments dealt with the effect of cadmium oxide on the worms developing from an initial infection. The present experiment was designed to learn the effect of this chemical on worms developing at reinfection.

Thirty mice, 3 months old, were divided equally, on the basis of sex and weight, into 3 groups. The 10 mice of Group I (experimentals) and the 10 mice of Group II (infected controls) were each given a stimulating infection with 200 *T. spiralis* larvae in an attempt to produce immunity, while the 10 mice of Group III were kept as non-infected controls. Twenty-eight days later, the mice of Group I were placed on the medicated feed, while those of Groups II and III were given the non-medicated feed. The mice of the 3 groups were kept on the respective feeds until necropsy, or for a total of 9 days. Two days after being placed on the special feeds, or 30 days after the stimulating infection, the mice of the 3 groups were each given a challenging infection of 400 *T. spiralis* larvae. Seven days later, the animals were killed and a count was made in each of the number of adult *T. spiralis* present in the small intestine.

TABLE I — *The number of adult* T. spiralis *recovered 7 days after a single infection with 200 larvae from mice fed cadmium oxide and from controls*

	Experimentals			Controls		
	Group I (CdO 48 hrs. prior to infection to necropsy)	Group II (CdO at infection to necropsy)	Group III (CdO 48 hrs. after infection to necropsy)	Group I (Non-medicated feed)	Group II (Non-medicated feed)	Group III (Non-medicated feed)
No. mice	8	8	8	8	8	8
Ave. no. adults	40.5	63.1	80.1	115.1	102.6	122.6
Range in no. of adults	18-81	29-104	39-104	88-166	68-124	85-155
Percentage dev. of adults	20.3	31.6	40.1	57.6	51.3	61.3

TABLE II — *The number of adult* T. spiralis *recovered 9 days after a single infection with 200 larvae from mice fed cadmium oxide and from controls*

	Experimentals			Controls		
	Group I (CdO 48 hrs. prior to infection to necropsy)	Group II (CdO at infection to necropsy)	Group III (CdO 48 hrs. after infection to necropsy)	Group I (Non-medicated feed)	Group II (Non-medicated feed)	Group III (Non-medicated feed)
No. mice	8	8	8	8	8	8
Ave. no. adults	27.1	46.9	89.7	154.9	147.9	141.2
Range in no. of adults	5-49	27-80	47-152	133-174	124-176	120-178
Percentage dev. of adults	13.6	23.5	44.9	77.5	74.0	70.6

TABLE III — *The estimated number of larvae recovered 30 days after a single infection with 200* T. spiralis *larvae from mice fed cadmium oxide and from controls*

	Experimentals			Controls		
	Group I (CdO 48 hrs. prior to infection to 10th day after infection)	Group II (CdO at infection to 10th day after infection)	Group III (CdO 48 hrs. after infection to 10th day after infection)	Group I (Non-medicated feed)	Group II (Non-medicated feed)	Group III (Non-medicated feed)
No. mice	8	8	8	8	8	8
Ave. no. larvae	3,996	7,610	13,408	33,475	39,065	33,282
Range in no. of larvae	132-24,600	2,060-19,720	6,340-28,600	22,000-54,000	19,800-65,000	15,260-66,800
Ave. ratio to no. larvae given at infection	19.9x	38.0x	67.0x	167.4x	195.3x	166.4x

Two special control groups, the viability controls (5 mice), and the persistency controls (10 mice), were also used in this experiment. The viability controls were given the stimulating infection and sacrificed 7 days later for adult worm counts. The number of adults recovered was that expected from the dose, proving the viability of the larvae used to infect the mice of Groups I and II. The persistency controls received only the stimulating infection. Five were fed the medicated feed and five were fed the non-medicated feed during the period that these special feeds were given to the mice of Group I and Groups II and III, respectively. The 10 persistency controls were sacrificed for adult worm counts at the same time the mice of Groups I, II, and III were sacrificed (i.e., 37 days after the stimulating infection). No adult worms were recovered from the persistency controls, so that it is safe to assume that those recovered from Groups I and II had developed from the challenging infection given 7 days before necropsy.

TABLE IV — *Showing the number of adult worms recovered from mice of Groups I, II, and III 7 days after a challenging infection of 400* T. spiralis *larvae. The mice of Groups I and II had received one prior stimulating infection (200 larvae)*

	Group I (Experimentals; stimulated, and medicated)	Group II (Infected controls; stimulated, but not medicated)	Group III (Non-infected controls; not stimulated, and not medicated)
No. mice	10	10	10
Ave. no. adults	21.7	220.6	213.3
Range in no.	4-45	181-272	171-294
Percentage dev.	5.4	55.1	53.3

The numbers of adult worms recovered from the mice of Groups I, II, and III are listed in Table IV, which shows that the average number of adults recovered from the experimental mice (21.7) is about 1/10th that recovered from the infected controls (220.6) and the non-infected controls (213.3).

DISCUSSION

As shown above, cadmium oxide (0.015%) in pelleted feed, given by 3 different feeding schedules, caused a striking reduction in the number of *T. spiralis* recovered from the mice at intervals after a single infection (Tables I, II, III). It is evident, therefore, that the action of the chemical is not dependent upon its presence in the tissues prior to invasion by the parasite. However, the fact that, in all cases, greater reductions occurred in the mice placed on the medicated feed before infection than in those given the same feed starting 2 days after infection may indicate that the chemical is most active against the pre-adult forms of the parasite. This should be investigated further in experiments designed to provide the medicated feed to all experimental groups for the same period of time.

The striking reductions in the numbers of larvae recovered from the treated mice (Table III) probably were brought about by the reduction in the numbers of adult worms (Table II), and not by action(s) of the chemical against the migrating and encysting larvae. There is also a likelihood that the chemical reduced the reproductive potential of the females that persisted in the intestine, but this was not determined in the present study.

The greatest reduction in the number of *T. spiralis* recovered in this study occurred in the previously infected mice placed on the medicated feed 48 hours before being reinfected (Table IV). The number of worms recovered 7 days after reinfection was about ¼ that recovered at the same period from the medicated mice given a single infection of the same size (Table I). At first glance this would seem to indicate that the difference was due entirely to the development of acquired immunity in those previously infected. However, this was not the case, since the nonmedicated mice given the same prior infection failed to develop a demonstrable acquired immunity. This failure, in fact, was expected from the design used, since the resulting immunity is feeble and can not be demonstrated in this way (Hendricks, 1950; and others). It is clear, therefore, that neither the chemical alone nor the feeble acquired immunity alone could have accounted for the striking reduction observed, and thus the effect was due to the combined action(s) of both factors. It seems likely that the humoral and cellular reactions, known to be associated with the immunity produced following a single infection (Larsh and Race, 1954; Larsh *et al,* 1956), interfered with the development of the worms to the extent that they were more susceptible to the action(s) of the chemical.

The results of this study in mice, showing a striking effect of cadmium oxide on *T. spiralis,* encourage the testing of this chemical against this parasite in swine. If, as seems likely from the above, continuous feeding of cadmium oxide to swine were shown to prevent the persistency of large numbers of adult *T. spiralis,* thereby resulting in only light muscle infections, the procedure might prove valuable in the control of human trichinosis. Since the severity of the disease is related to the number of infective larvae ingested, one might expect in time to prevent acute human cases by controlling in this practical way the level of infection in the important reservoir. Fortunately, the stage is set for the testing of cadmium oxide against *T. spiralis* in swine, since this chemical has been shown to meet the requirements of an ideal swine anthelmintic. It can be given conveniently in all types of rations, and has a unique safety feature, emesis occurring when toxic amounts are consumed (Burch and Blair, 1955).

SUMMARY

Medicated feed in pellet form containing 0.015% cadmium oxide was shown, by various feeding schedules in mice, to produce a striking reduction in the number of adults and larvae of *T. spiralis* recovered after a single infection. An even greater effect was shown in the reduction of adult worms recovered from mice after 1 reinfection. The results encourage the testing of this chemical against *T. spiralis* in swine.

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RESEARCH NOTE

A METHOD FOR COLLECTING ECTOPARASITES FROM BIRDS

While working with the ectoparasite populations on small mammals, we developed a technique (Cook, 1954, Ent. News **65**: 35–37) with which we were able to collect the total populations of the lice, fleas and mites that were on these hosts. Since little data of a quantitative nature are available on the ectoparasite populations on birds, we used this technique on a small series of birds to determine whether it would be practical or not, with this group.

In order to obtain reliable data on either a qualitative or quantitative basis, extreme care must be exercised at all times to prevent the loss of parasites or the contamination of the host with parasites from other hosts. When the bird is taken, it should be placed immediately in a polyethelene home freezer bag and the top securely closed. A small amount of ether (about 2 ml for small birds) should be placed in the bag to kill the parasites and the host if it is not already dead.

The bird is then skinned. This operation should be carried out on a clean sheet of white paper so that any parasites that accidentally drop off may be saved. Each bird is skinned on a separate sheet to prevent contamination from parasites lost from previous birds. The skin is then dried, sealed in the plastic bag and stored until it is convenient to examine the skin for parasites.

For a bird the size of an English sparrow the skin is placed in a 125-ml Erlenmeyer flask and covered with a solution of 1% Trypsin (4× U.S.P. Pancreatin) buffered to a pH of about 8.3 with Na_2HPO_4. Enough solution for 6 or 7 birds may be made up as follows: 500 ml of distilled water, 15 g anhydrous sodium phosphate dibasic (Na_2HPO_4), 6 g Trypsin (4× U.S.P. Pancreatin. The skin is then placed in an oven at 38° C for a period of from 12 to 24 hours. When the skin is removed from the oven about 15 g KOH is added to dissolve the feathers and the remainder of the skin. The resulting mixture is brought to a boil and then allowed to cool for at least a few minutes and then brought to a boil a second time. The resulting liquid is poured through a 200-mesh bronze screen (folded into a conical form). The residue on the screen is washed with distilled water to remove any remaining soap or pigment. The screen is inverted in a glass petri dish and the ectoparasites washed off by a stream of water from a washing bottle. The screen is examined under a dissecting microscope for any parasites that were not washed off into the petri dish. The contents of the petri dish are examined under a dissecting microscope and the parasites removed.

The arthropod parasites are largely cleared by this method and aside from some manipulation to remove the dissolved body contents, are ready for staining and mounting in the appropriate media.—JAMES R. BEER AND EDWIN F. COOK, *Department of Entomology and Economic Zoology, University of Minnesota, St. Paul 1, Minnesota.* (Paper No. 3769, Scientific Journal Series, Minnesota Agricultural Experiment Station.)

THE OXYURID NEMATODES OF RODENTS
I. THE GENUS *CITELLINA* PRENDEL

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The genus *Citellina* was erected by Prendel (1928) for the reception of *C. dispar* which he recovered from *Citellus suslica* at Odessa. Manter (1930) described *Citellina marmotae* from *Marmota monax canadensis* in Maine and pointed out that *Oxyuris triradiata* Hall, 1915, from Colorado ground squirrels should be referred to Prendel's *Citellina*. Schulz (1930) described *C. petrowi* from *"Pteromys volans"* in northern Dvinsk, Russia, and arrived at the same conclusions as Manter concerning the allocation of *Oxyuris triradiata* Hall. Subsequently, other species have been described and referred to this genus; these are *C. levini* Li, 1933, from *Sciurus vulgaris* at Peiping; *C. alpina* Jettmar and Anschau, 1951, from *Arctomys marmota* (= *Marmota marmota* L.) in the Austrian Alps; and *C. alatau* Spasski, Rjikov and Sudarikov, 1950, from *Marmota menzbieri* in European Russia. Study of the descriptions of these species reveals some rather interesting differences. Prendel (1928) reported that *C. dispar* possessed a long thin spicule and a gubernaculum while Manter reported that *C. marmotae* from the American woodchuck possessed a spicule but no gubernaculum. Shulz described *C. petrowi* as having a spicule and a gubernaculum and differentiated it from *C. dispar* Prendel by its possession of a spicule which was about 1/6 of the length of that described for the type species of the genus. In *Oxyuris triradiata* Hall described and figured a short stout spicule resembling that structure which Prendel referred to as the gubernaculum in *C. dispar*. Hall did not describe a gubernaculum in *Oxyuris triradiata*. The various authors of the species names *C. levini, C. alpina,* and *C. alatau* have reported both a spicule and gubernaculum to be present in their materials and have differentiated their species from *C. marmotae* Manter and *C. triradiata* (Hall) primarily on these grounds. In an abstract Dubinina (1947) listed *C. dispar* Prendel as a synonym of *C. triradiata* (Hall).

Through the kindness of Dr. Robert Rausch a sizable collection of oxyurids from Alaskan marmots and ground squirrels has become available. In addition I have collected a considerable amount of material from ground squirrels in the Western U. S. and from woodchucks in the Eastern U. S. Careful study of the *Citellina* in this collection has convinced me that only a single species of *Citellina* is represented. In order to determine more exactly the status of *C. marmotae* Manter and *C. triradiata* (Hall) the type specimens of these species were borrowed from the U. S. National Museum. Appreciation is expressed to Dr. E. W. Price who made these and some other pertinent collections available for study and to Dr. David E. Davis, Dr. Marietta Voge, and Mr. William Reeder who abetted my collecting of marmots and ground squirrels in the United States.

OBSERVATIONS

Study of the type specimen of *C. marmotae* reveals that a gubernaculum is present. This structure is not easily seen because it is poorly sclerotized. The spicule is more readily seen in the specimen and was quite accurately figured by Manter

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(1930). The accessory bursal membranes are quite similar to those structures as pictured for *Oxyuris triradiata* by Hall (1915). Study of the type male of *Oxyuris triradiata* Hall indicates that the structure which Hall called a spicule is a gubernaculum plus the terminal portion of a weakly sclerotized spicule. Hall's figure is quite accurate but his interpretation of the structures has resulted in some confusion. The gubernacula of the type males of *C. marmotae* Manter and *C. triradiata* (Hall) are shown in Figures 1 and 2 (Plate I). These may be compared with males from some other North American hosts and localities shown in Figures 3 to 6. Hall (1915) gave the spicule length of *Oxyuris triradiata* as 45 to 50 microns. However, in 1 of the 2 males in the type material there is readily seen a weakly sclerotized spicule which is 223 microns in length. The proximal portion of this spicule is the most sclerotized part and is shown in Figure 14. In males of *Citellina* collected by Dr. Rausch from a single individual of the hoary marmot the spicule ranges from 140 to 340 microns in length. The spicules of the types of both *Oxyuris triradiata* Hall and *Citellina marmotae* Manter fall within this range. In his description of *C. dispar* Prendel gave the spicule length as 1.64 mm. However, study of Prendel's figure shows that there is an obvious typographical error in the text, the actual measurement probably having been 0.164 mm. The transverse fold on the dorsal side of the "bursa" of *Oxyuris triradiata,* figured by Hall (1915), is an inconstant character found in my material from *Citellus* and *Marmota.* It appears to be an artifact of fixation. The second postanal median papilla described by Manter (1930) in the male of *C. marmotae* may be seen with difficulty in the type male of this species. It may also be seen in some, but not all, male *Citellina* from *Marmota marmota broweri* and *Citellus leucurus* subsp. Study of sectioned material has shown that it is not a true papilla, and it is considered to be a variable character.

The original description of *Oxyuris triradiata* did not include data on the eggs of the species and Hall stated that none of his specimens were gravid. Curiously, my material from several *Citellus leucurus* subsp. taken at Lovejoy Butte, 18 miles east of Palmdale, California, contains many females contaning no eggs. On the other hand, some females, of approximately the same size and taken from the same host individual, are gravid. The eggs of worms from *Citellus* spp. from California and Alaska are identical with those of worms from the marmot material available, including the types of *C. marmotae* Manter. The prominent vulvar labia found in *Citellina* females vary considerably. Specimens collected by Dr. Rausch from the hoary marmot show all of the variations in these structures corresponding to the descriptions of labial anatomy in all the species of *Citellina.* With the exception of *C. levini,* the size of the eggs and other characteristics described for females of the species of *Citellina* do not lend themselves to specific differentiation. *C. levini* Li was described as lacking the polar filaments described for the egg of *C. marmotae* Manter. This would seem to be the only character which distinguishes these two species. It is my opinion that a careful restudy of *C. levini* will show that polar filaments are present on the egg and, for the present, it is regarded as a *species inquirenda.*

In the descriptions of worms belonging to the genus *Citellina* there has been a most astonishing confusion. Hall (1915) apparently failed to see the male spicule; Prendel (1928) evidently failed, or did not have an opportunity, to correct the misplacing of a decimal point in his published measurements of the male spicule; Man-

ter (1930) did not recognize the presence of a gubernaculum. Shulz (1930) presented the only accurate description of a *Citellina* with very fine illustrations; it is unfortunate that his work appeared in an almost inaccessible publication. On the basis of the present study it is apparent that there are no morphological characters which can be used to separate *Oxyuris triradiata* Hall, *Citellina dispar* Prendel, *C. marmotae* Manter, *C. petrowi* Shulz, *C. alpina* Jettmar and Anschau, and *C. alatau* Spasski, Rjikov, and Sudarikov. It is concluded that there is a cosmopolitan distribution of a single species of *Citellina* in ground squirrels and marmots. The correct name for this species is *Citellina triradiata* (Hall). *C. levini* Li is probably synonymous with *C. triradiata* (Hall) but the information available is insufficient to establish this.

Of special interest is the apparent independence of climate which is shown by this species. It is found in hosts from the moist, forested eastern and northern U. S., the desert areas of southern California, subarctic localities in Alaska, the steppes of Russia, and the Austrian Alps.

The hosts involved are probably not so isolated as has been thought previously. With reference to the marmots Rausch (1953) pointed out that "each form grades into the next, demonstrating a cline which extends from Switzerland and south Germany across Siberia into North America."

TABLE I.—*Hosts and geographical distribution of* Citellina triradiata *(Hall)*

Host	Locality	Reference
Citellus leucurus cinnamomeus (= *Ammospermophilus l. cinnemomeus*)	Colorado	Hall, 1916 and present paper
C. leucurus subsp.	California	present paper
C. lateralis (= *Callospermophilus lateralis*)	Colorado	Hall, 1916
C. suslica	Odessa (Russia)	Prendel, 1928
C. citellus	Roumania	Nitzulescu, 1946
C. undulatus ablusus	Southern Alaska	present paper
C. undulatus barrowensis	Northern Alaska	present paper
"Pteromys volans"	Dvinsk (Russia)	Shultz, 1930
"Arvicola terrestris"	Kazakhstan (Russia)	Agopova, 1953
Marmota monax canadensis	Maine	Manter, 1930 and present paper
M. monax monax	Pennsylvania	present paper
M. m. rufescens	Wisconsin	present paper
Marmota sp. (probably *M. m. monax*)	Maryland	present paper
Marmota sp. (probably *M. m. preblorum*)	Connecticut	present paper
M. marmota broweri	Northern Alaska	present paper
M. marmota baibacina	Kazakhstan (Russia)	Agopova, 1953
M. menzbieri	Talass Altai (Russia)	Spasski *et al.*, 1950
M. marmota (= *"Arctomys marmota"*)	Austrian Alps	Jettmar and Anschau, 1951

It seems appropriate to present a list of hosts for *C. triradiata*. All names of North American species have been checked with Miller and Kellogg (1955), except for the Alaskan forms to which the nomenclature of Rausch (1953) has been applied (Table I).

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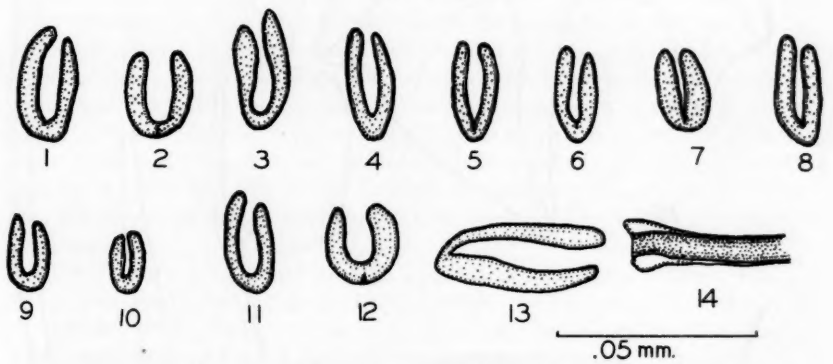
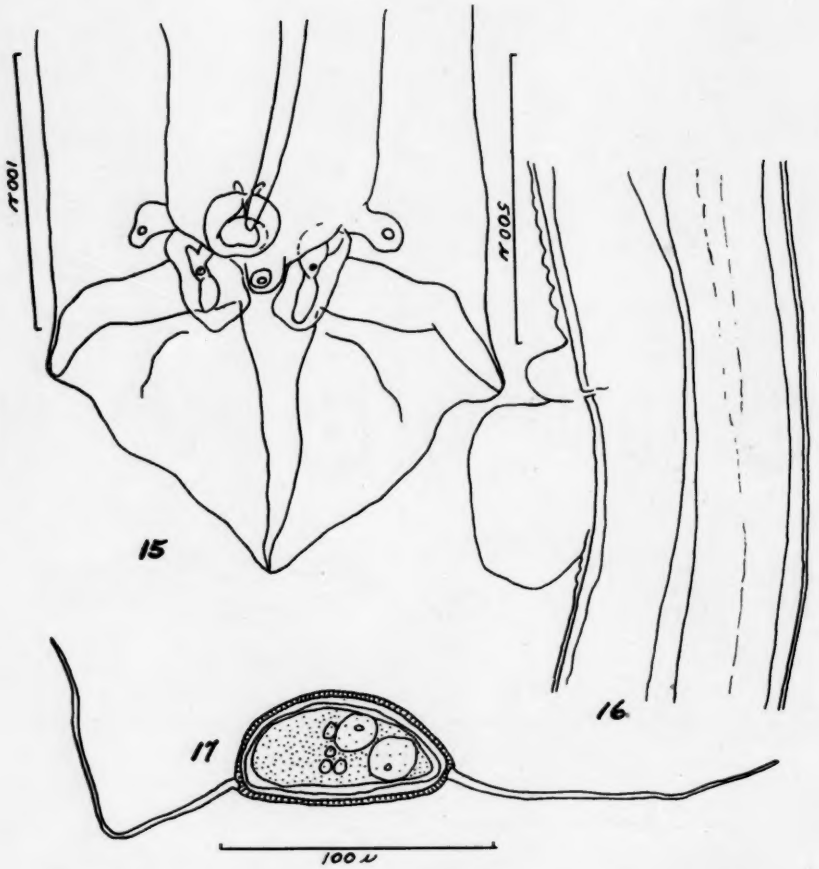
PLATE I



1. Gubernaculum of type male, *Citellina triradiata* (Hall), U.S. Natl. Mus. Helm. Coll. No. 16582.
2. Gubernaculum of type male, *C. marmotae* Manter, U.S. Natl. Mus. Helm. Coll. No. 8081.
3. Gubernaculum of male *C. triradiata* (Hall) from *Marmota* sp. (Maryland), U.S. Natl. Mus. Helm. Coll. No. 46435.
4. Same from *Citellus leucurus* subsp. (California).
5–8. Same from *Marmota monax monax* (Pennsylvania).
9. Same from *M. monax rufescens* (Wisconsin).
10. Same from *Citellus undulatus ablusus* (Alaska).
11. Same from *C. undulatus barrowensis* (Alaska).
12–13. Same from *Marmota marmota broweri* (Alaska).
14. Proximal portion of the spicule of type male, *C. triradiata* (Hall).

PLATE II



15. Caudal end of male *C. triradiata* from *Marmota monax rufescens.*
16. Vulvar region of female *C. triradiata* from *M. m. rufescens.*
17. Egg from female sketched in 16.

GYRODACTYLUS EUCALIAE N. SP. (TREMATODA: MONOGENEA) FROM THE BROOK STICKLEBACK, *EUCALIA INCONSTANS**

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A new species of *Gyrodactylus* was observed on the skin, gills, and fins of the brook stickleback from the English Coulee, Grand Forks, North Dakota during the summers of 1953–1955. Host-specificity is usually striking in the monogenetic trematodes and since this parasite was not observed on any other species of fish from this body of water or the nearby Turtle River, it was studied in detail and is here described as a new species, *G. eucaliae*.

Ether chloretone, chloroform, or menthol at 0.5%, or urethane or nembutal at 0.1 to 0.5%, in aqueous solution, were used in recovering the intact worms from the host. The best solutions proved to be 0.5% urethane and 0.5% nembutal. One is cautioned concerning the carcinogenic properties of urethane (Wood, 1956). A convenient field method of collecting the parasites in large numbers consisted of placing the fish in a bottle of 0.5% nembutal or urethane; the parasites left the host within 3 to 5 minutes and the hosts were discarded; the fish survived about 5 minutes. Parasites so obtained remained suitable for study for several hours. They were concentrated by sedimentation and fixed in hot 5% glycerine–5% formalin solution. Living worms were studied without the use of anesthetics but were freed from dead fish under refrigeration. The anchors and marginal hooklets were found to be anisotropic with polarized light. The armature was studied in part by allowing the soft parts of the worm to decompose under a vaseline-sealed coverslip and it was found that 16% KOH dissolved away the anchors, but not the ventral bar, dorsal bar, or the haptoral shield; this aided in observation of the haptoral armature.

Killed worms were affixed to slides with Haupt's Adhesive (Hargis, 1952) or PVA fixative-adhesive (Hoffman, 1954) and then were stained with coelestin blue B, (Demke, 1952), Semichon's carmine, borax carmine, Harris' hematoxylin, picro-carmine, chlorazol black E, and silver impregnation (Arcadi, 1948). They were dehydrated in cellosolve, cleared in xylol, and mounted in permount. The hooks were most consistently stained selectively with picro-carmine. Both chlorazol black E and Simichon's carmine were selective in staining the dorsal bar, the ventral bar, and haptoral shield in 10 minutes. Arcadi's silver impregnation method was found to be best for the head organ; it also gave selective staining for the ventral bar, dorsal bar, haptoral shield, and the testis. All measurements are in millimeters and based on 10 specimens except where indicated otherwise.

Gyrodactylus eucaliae n. sp.

Description: The body is small, cylindrical and fusiform 0.445 (0.387–0.590) by 0.088 (0.066–0.127) (Fig. 1). Cuticle thin and smooth. Opisthohaptor, 0.073–0.09 by 0.083–0.110, a concavo-convex disc opening ventro-posteriorly; armed with 2 anchors, 2 bars, haptoral shield, and 16 marginal hooklets. Anchors moderately stout, 0.069 (0.067–0.071) from base to point of greatest curvature and 0.048 (0.045–0.051) from base to the anchor point with slightly curved roots

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* This study was undertaken in partial fulfillment of the requirements for the M.S. degree by the senior author (1955). This species was described as *G. incisura* in his thesis.

and strongly recurved tips (Fig. 1, 2); a "split" 0.044 long and 0.002 at the widest can be seen laterally in some specimens. Ventral bar, 0.026 to 0.031 by 0.007, relatively stout and gently curved. Dorsal bar delicate, 0.018–0.034 by 0.002, with a notch at the midpoint of the posterior edge. Marginal hooklets 0.046 (0.041–0.049) long with long thin shafts with a small proximal bulb (Fig. 2). Haptoral shield, 0.018–0.02 by 0.017–0.019, thin and always present; it is not an

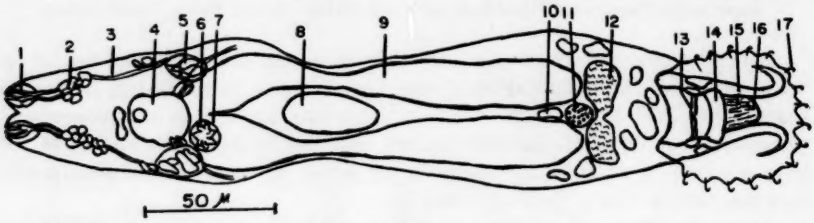

FIGURE 1. Composite drawnig of *Gyrodactylus eucaliae* n. sp.

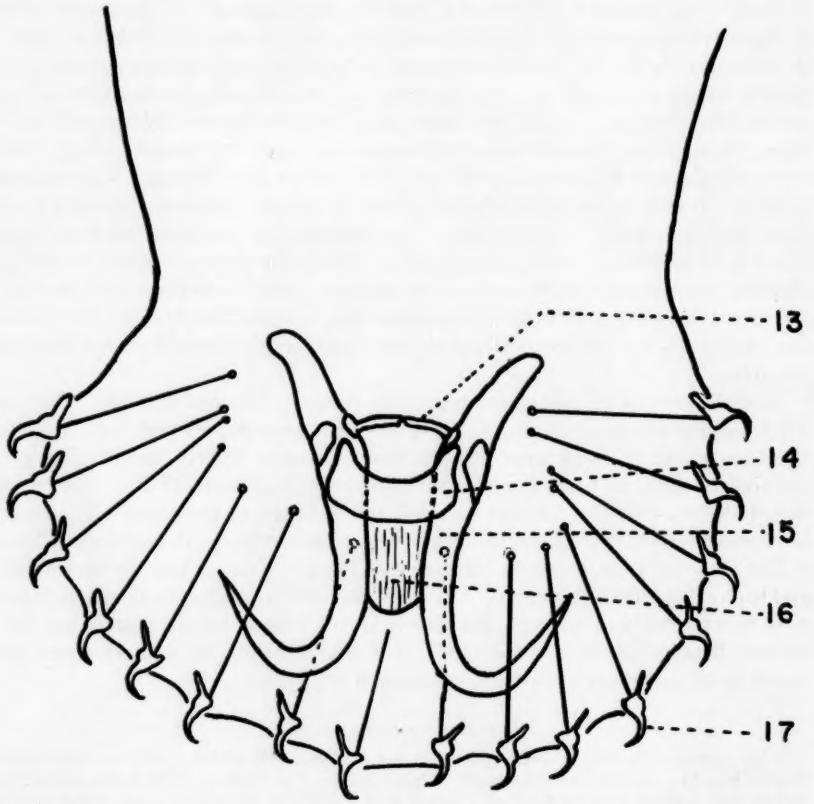

FIGURE 2. Composite drawing of Opisthohaptoral armament of *G. eucaliae* traced from photomicrographs.

Explanation of Figures: 1, Adhesive organ; 2, Coiled tubule; 3, Connecting tubule; 4, Pharynx; 5. Cephalic gland; 6, Stylet; 7, Cirrus pouch; 8, Embryo; 9, Intestinal crura; 10, Ootype; 11, Ovary; 12, Testis; 13, Dorsal bar; 14, Ventral bar; 15, Anchor; 16, Shield; 17, Marginal hooklet.

extension of the ventral bar as has been reported for some other species, but appears, instead, to be an independent structure that lies upon the ventral bar dorsally and clamps onto the surface of the anchors. The head organ consists of a pair of antero-lateral, papillate organs which lead into a set of globular vesicles lying just antero-lateral to the pharynx; from this structure connecting tubules extend posteriorly; their exact origin could not be determined. Dorso-lateral to the pharynx are 2 other vesicular structures termed "cephalic gland" by Mueller and Van Cleave (1932) and "prostate gland" by Yin and Sproston (1948); in our specimens tubules could be seen connecting these structures to the pharynx—at intervals this "gland" fills with fluid and discharges, like a contractile vacuole, into the pharynx. The bilobed pharynx, 0.037 (0.034–0.041) by 0.038 (0.036–0.041) leads directly into the intestine which immediately bifurcates into 2 large crura. Testis, 0.018–0.029 by 0.044–0.068, bilobed, sometimes gives the appearance of 2 testes, situated at posterior ends of intestinal crura. The cirrus, 0.01–0.014, has 7 stylets and no large spine. Ovary oval and anterior to the testis. Ootype larger, 0.024, and posterior to ovary. Vitellaria 2 irregular groups of lightly staining bodies lateral to the posterior ends of the intestinal crura (not shown in drawing). Often large embryo in utero with second embryo often contained within first.

Under phase microscopy cells were noticed in the intestinal crura; the worms were crushed with gentle pressure to rupture the intestine and release the cells which were cultured in broth and on a nutrient slant at 4–6° C. Moderate growths were obtained, which were found to be Gram-positive motile rods and yeast-like mold, but were not otherwise identified. They were a constant intestinal flora of the gyrodactylid.

Host: *Eucalia inconstans* (Kirtland), Brook Stickleback.
Location: Gills and skin.
Locality: English Coulee, Grand Forks, North Dakota.
Paratype: U. S. National Museum Helm. Coll. No. 55619.

Comparison:

A brief comparison is made here of those species of *Gyrodactylus* found in North America. The dorsal bar is straight and narrow with a notch at the midpoint in *G. eucaliae.* On the other hand the dorsal bar is absent in *G. cylindriformis,* Mueller and Van Cleave, 1932, and in *G. fairporti,* Van Cleave, 1921. This new species has a shield with longitudinal striations similar to *G. stequrus* Mueller 1937, and *G. stephanus,* Mueller, 1937. However, the cirrus pouch of *G. eucaliae* is unlike those of *G. stequrus* and *G. stephanus,* both of which have a spine and 8 stylets. The shield lacks striations in *G. elegans,* Nordmann, 1832, (Mueller, 1936) and the shield is absent in *G. medius,* Katheriner, 1894, (Van Cleave, 1921). *G. spathulatus,* Mueller, 1936, has a shield so peculiarly characteristic in its spatula-shape that it can hardly be mistaken for any other known species of *Gyrodactylus. G. funduli* Hargis, 1955, unlike any of the above, has anchors with truncated roots, and *G. prolongis,* Hargis, 1955, is the only one which has an unusual skirt-like accessory holdfast on the peduncle.

Pathogenicity:

Yin and Sproston (1948) found that the fan-tailed goldfish of the black and golden varieties were killed by gyrodactyliasis. Death of the infected fishes was thought to be due ultimately to the hyper-secretion of mucus on the gill surfaces, brought about by the irritation due to the large population of gyrodactylids. A very interesting fact that they mention is the presence of another pathogen. They found an exceedingly heavy infestation of a peritrichous ciliate, probably a species of *Trichodina,* which was not only browsing on the gill surfaces, but which also fastened itself as an epiparasite on the gyrodactylids.

Van Cleave (1921) also reported fatal gyrodactyliasis of the bullhead, *Ameiurus melas,* at Fairpoint Biological Station of the U. S. Bureau of Fisheries, Iowa. The fish were dead in less than 24 hours after their removal to a running water aquarium

in the laboratory. Careful examination revealed no cause other than the presence of small trematodes of the genus *Gyrodactylus*. He also mentioned a case in which a species of *Gyrodactylus* was discovered on the gills of the rainbow trout in the New York state hatchery at Cold Springs Harbor, Long Island. In many instances their presence in large numbers caused the gills to become shriveled and functionless. He concluded that especially favorable conditions for heavy general infestation result when fishes are crowded in ponds and pools.

There has been no evidence of irritation or fatality that could incriminate gyrodactylids on *Eucalia inconstans* except under unusual conditions in aquaria. A *Trichodina* representative was found also on the stickleback infected with gyrodactylids.

Host Specificity:

Hargis (1953) gives evidence that a very high degree of host specificity exists among the monogenetic trematodes; this specificity is so marked that it is possible to make a fairly accurate identification of the hosts by an analysis of their ectoparasitic trematodes.

One lot of sticklebacks was kept together with fatheads, *Pimephales p. promelas*, for a period of over 2 months. During the first month the gyrodactylids and the ectoparasitic protozoan, *Trichodina* sp. were found in abundance on the sticklebacks. After the first month an occasional gyrodactylid could be recovered and toward the end of the second month the fish were usually entirely free of them. The only ectoparasites found on the fatheads during this time were *Dactylogyrus* sp. and *Ergasilus* sp. Their disappearance from the natural host suggests an actively acquired immunity which has not heretofore been mentioned in connection with species of *Gyrodactylus*.

Culture of Gyrodactylus:

No record could be found of an attempt to culture *Gyrodactylus* artificially. It was believed that if the period of gestation could be determined their approximate density on the host population could then be predicted. Tissue culture media (Syverton, Scherer, and Elwood, 1954) used to grow cancer cells, was diluted 1:4 at 4–6° C, but no new embryos were produced, although the worms remained alive for a maximum of 1 month. It seemed that the embryo died before the parent died. Normally the embryo can be seen undergoing muscular activity even before birth, but after 2 to 3 weeks of culture in this medium the embryo, even though mature, lost its muscular activity. The medium seemed to lack a factor necessary for parturition. An attempt to induce abortion using acetylcholine and adrenalin was made but this proved to be unsuccessful. Another attempt at culturing the trematode was made by using macerated *Eucalia* filtered through Berkefeld filters for sterility but this was unsuccessful.

SUMMARY

1. *Gyrodactylus eucaliae* n. sp. from the brook stickleback, *Eucalia inconstans*, is described and compared with 9 other species previously reported from North America.

2. The anchors and marginal hooks have been found to be anisotropic. The anchors, but not the ventral bar, dorsal bar, and the apron are soluble in 16% KOH.

3. Placing the fish hosts in 0.5% nembutal or urethane was found to be the best method of getting mucus-free parasites rapidly.

4. A Gram-positive bacillus and yeast, species unidentified, were found to be constant intestinal organisms in *Gyrodactylus eucaliae*.

5. Diluted tissue culture medium was successful for maintenance of the worms at 4° to 6° C, but not for reproduction. Macerated filtered *Eucalia* medium was unsuccessful for maintenance and reproduction.

6. The continued decline in intensity of infections on fishes in aquaria suggests the possibility of an actively acquired immunity by the host, *Eucalia inconstans*.

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RESEARCH NOTE

AN UNSUCCESSFUL ATTEMPT TO INFECT BATS WITH *SCHISTOSOMATIUM DOUTHITTI*

In an attempt to study some of the factors influencing the production of hermaphroditic schistosomes, an experiment was designed using the bat as a host and varying the environmental temperature. Since the bat is heterothermic the environmental temperature of the schistosomes could thus be controlled.

Following the method described by Constantine (1952) for maintaining the Mexican freetail bat, *Tadarida mexicana*, fair success was experienced in the maintenance of a smaller species, *Myotis austroriparius* (identified by W. L. Jennings).

As a preliminary experiment, eleven bats were kept in a large drying oven with 3 large shallow pans of water. The oven was slightly ventillated and kept at 34–35° C. Three or 4 times per day the bats were taken out and taught to eat small June bugs from the floor of their containers. Six bats were exposed to 40 *Schistosomatium douthitti* cercariae, applied to areas with little hair (feet, wings, etc.) by means of the hair loop technique.

Autopsies were performed 17, 20 (2 bats), 53, 54, and 60 days after exposure. All were negative.

The wing of a 7th bat was stretched across the stage of a compound microscope so that the activity of several cercariae could be observed in a drop of water placed on the bat's wing. No apparent attempt to penetrate was made during 2 hours of continuous observation.

Since the skin may be the critical barrier to schistosome infection, the author plans to expose other bats by injecting cercariae into the abdominal cavity.—DONALD W. DERY, *Department of Biological Sciences, Florida State University.*

ON THE INTRASPECIFIC AND INTERSTRAIN VARIATIONS OF THE MALE SEXUAL GLANDS OF *SCHISTOSOMA JAPONICUM*

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Most textbooks of parasitology state that *Schistosoma haematobium* has 4 to 5 testes, arranged in a cluster, that *S. mansoni* has 6 to 9, also clustered, and that *S. japonicum* has 7, arranged in a single column. Garcia (1953) stated that 92% of the males of *S. japonicum*, studied in the Philippines, had 7 testes, and that the remainder had 8. This evidence of variability encouraged the present study, which places special emphasis on a comparison of the males of the 4 geographic strains reported from the Chinese mainland, Formosa (China), Japan, and the Philippines (DeWitt, 1952, 1954; Hunter *et al*, 1952). It was of particular interest to determine whether or not the zoophilic strain (Hsü *et al*, 1956) differed morphologically from the anthropophilic strain from the other areas.

MATERIALS AND METHODS

A total of 4,064 males of *S. japonicum* from infected animals was examined. Of these, 1,727 specimens were of the Formosan strain, from the infected snail, *Oncomelania formosana*, from Changhua, Formosa, China; 1,434 came from bisexual infections, and 293 from unisexual infections. The specimens of the Chinese strain consisted of 345 specimens, all from bisexual infections. The cercariae were obtained from *O. hupensis* originating in Kashing, Chekiang, China. Specimens of the Japanese strain consisted of 1,908 males, with 684 from bisexual infections, and 1,224 from unisexual infections. The cercariae were obtained from *O. nosophora* from Kyushu, Japan. The specimens of the Philippine consisted of only 84 males from a single bisexual infection. The cercariae were obtained from *O. quadrasi* from Leyte, the Philippines.

The definitive hosts included dogs, cats, goats, water buffaloes, pigs, horses, monkeys (*Macacus cyclopis*), rabbits, guinea pigs, shrews, hamsters, rats, and mice. The age of the worms was not uniform, but all of them were mature. The worms were all alive when collected, and were fixed under a coverslip with Bouin's fluid and stained with alum carmine.

RESULTS

There were 4 types of variations in the sexual organs of the males of *S. japonicum*: (1) the arrangement of testes in the usual location, (2) the number of testes, (3) testes in unusual locations, and (4) hermaphroditic males.

(1) *Arrangement of Testes in the Usual Location.*

The arrangement of testes can be divided into 2 groups: (1) tandem (Fig. 1) and (2) non-tandem (Figs. 2–4). In extreme cases, the non-tandem testes can form a cluster, simulating the arrangement of testes in *S. mansoni* or *S. haematobium*. The over-all percentages of the two types of arrangement, in this species, are more or less equally divided (Table I).

In the Formosan, Chinese, and Japanese strains, the host-species can be divided into 2 groups on the basis of the arrangement of the testes of the parasite: (1) those having more specimens with tandem than non-tandem, and (2) those having fewer. In the Formosan strain, the hamster, mouse, and rabbit belong to the first group, and the cat and dog to the second. In the Chinese strain, the dog, hamster, and

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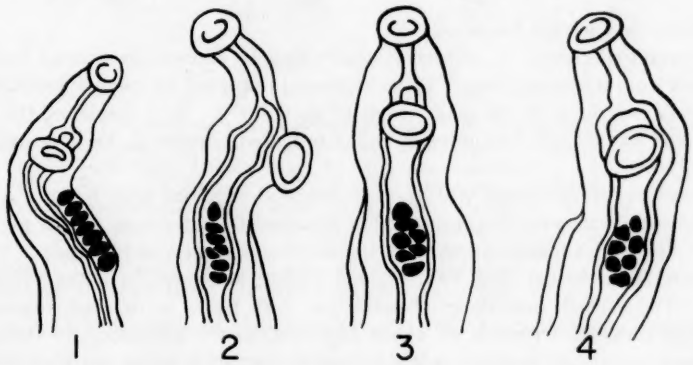
TABLE 1.—*The numbers and percentages of specimens with tandem or non-tandem testes in the males of the 4 geographical strains of* S. japonicum. *All the specimens were obtained from hosts infected with male and female cercariae*

Strain	Host (No. of Animals)	No. of Males Examined	Specimens With Tandem Testes		Specimens With non-Tandem Testes	
			No.	%	No.	%
Formosan	Hamster (7)	55	47	85	8	15
	Mouse (4)	128	69	54	59	46
	Rabbit (5)	204	147	72	57	28
	Cat (3)	27	8	30	19	70
	Dog (4)	160	32	20	128	80
	Others (34)*	860	413	48	447	52
	Total (57)	1434	716	50	718	50
Chinese	Dog (3)	113	66	58	47	42
	Hamster (5)	125	89	71	36	29
	Mouse (6)	45	25	55	20	45
	Cat (4)	58	20	34	38	66
	Other (1)*	4	0	0	4	100
	Total (19)	345	200	58	145	42
Japanese	Rabbit (5)	132	85	64	47	36
	Cat (4)	135	59	44	76	56
	Monkey (6)	312	65	21	247	79
	Others (6)*	105	55	52	50	48
	Total (21)	684	264	39	420	61
Philippine	Monkey (1)	84	40	48	44	52
TOTAL		2547	1220	49	1327	51

* The word "other" or "others" means buffaloes, goats, guinea pigs, horses, monkeys, pigs, rats, and shrews for the Formosan strain; a guinea pig for the Chinese strain; and dogs, hamsters, and rats for the Japanese strain. The number of animals or the number of the worms in these host-species is not large enough for making an analysis of the variance among the host-species.

mouse belong to the first group, and the cat to the second. In the Japanese strain, the rabbit belongs to the first group and the cat and monkey to the second. The F test shows that, in each strain, the difference in the testicular arrangement among specimens from different host-species in the same group is not significant, but that



FIGURES 1–4. *S. japonicum,* male, showing specimens with different types of the arrangement of testes.

FIGURE 1. Tandem type, Japanese strain, 112 days, from *Macacus cyclopis.*

FIGURES 2–3. Non-tandem type, Chinese strain, 87 days, from a dog.

FIGURE 4. Non-tandem type, cluster formation, Chinese strain, 22 days, from a dog.

it is significant in those specimens from different host-species of different groups. In the Philippine strain, as the specimens were obtained from only 1 monkey, it is impossible to evaluate the host influence on the arrangement of testes. It indicates, however, that both tandem and non-tandem types occur in this strain also.

While the host specificity seems to influence the testicular arrangement of the parasites on one strain, it is of interest to know also whether this arrangement will be of the same type, in one host-species, for all strains. Sufficient specimens of the Chinese, Formosan, and Japanese strains were obtained from the cat for comparison. From this host-species, in the Chinese and Formosan strains, there are fewer specimens with tandem testes than non-tandem, whereas in the Japanese strain there are more. It indicates, therefore, that in one host-species, the arrangement of the testes is not necessarily of the same type for all the strains.

(2) *Number of Testes.*

In addition to the most common 7-testes specimens, males with 1, 3, 4, 5, 6, 8, 9, and 14 testes have been found (Figs. 5–12). Further analysis shows that 90.5% of the specimens had 7 testes; 6.7% had 6; 1.2%, 5; and 1.1%,8. The specimens with other numbers of testes were all below 1%.

For convenience, the specimens have been divided into 2 groups: specimens with 7 testes, and specimens with the testis number *other* than 7. The Chi-square test shows that in the Formosan, Japanese, and Chinese strains, no significant difference in the number of testes was found among the specimens from different host-species. For the Philippine strain, the test was not made, because all the specimens were obtained from 1 monkey.

As the number of testes in the specimens from different host-species, infected with the same strain of the parasite, was not found to be significantly different, all the specimens of one strain were treated as a group. The percentages of the specimens with 7 testes in the four strains are: Japanese, 95.4; Chinese, 91.9; Formosan, 88.4; and Philippine, 77.4. The Chi-square test shows that the percentages of specimens with 7 testes are significantly different between all the strains, except in the case of the Chinese and Formosan.

(3) *Testes in Unusual Locations.*

In certain specimens, 1, or several, testes may be situated in unusual location, separated from the main group. These separated testes can be located anteriorly or posteriorly in relation to the main group (Figs. 13–17). In 1 specimen, the separated testis was located just anterior to the re-united portion of the intestinal ceca (Fig. 17).

Altogether, 36 specimens with testes in unusual locations were found. Among them, 23 specimens were Formosan strain, obtained from the dog, guinea pig, rabbit, and shrew; 8, Chinese strain, from the cat, dog, hamster, and mouse; 5, Japanese strain, from the cat, dog, monkey, and rabbit; and none from the Philippine strain. The over-all percentage of specimens with testes in unusual locations is low, being only 2.2. Indeed, in all the host-species, the percentage of specimens with testes in unusual locations is low, except in the rabbit which has been infected with the Formosan strain, and in this case the percentage is higher, *e.g.,* 9.8. It is of interest to note that in the rabbit infected with the Japanese strain, the percentage of specimens with testes in unusual locations is also low, only 1.5. As the number

of specimens with testes in unusual locations obtained from each host was very small, the "likelihood ratio test" was appropriate for testing the significance of the differences. The test showed that in the Formosan strain the number of specimens, obtained from the rabbit, with testes in unusual locations, was significantly different from that obtained from other hosts, and, that in the rabbit, the specimens of

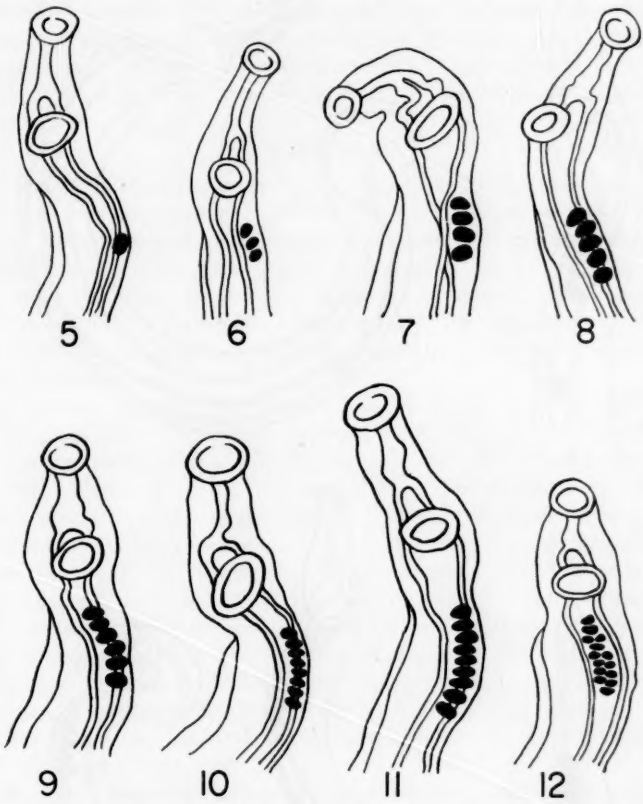


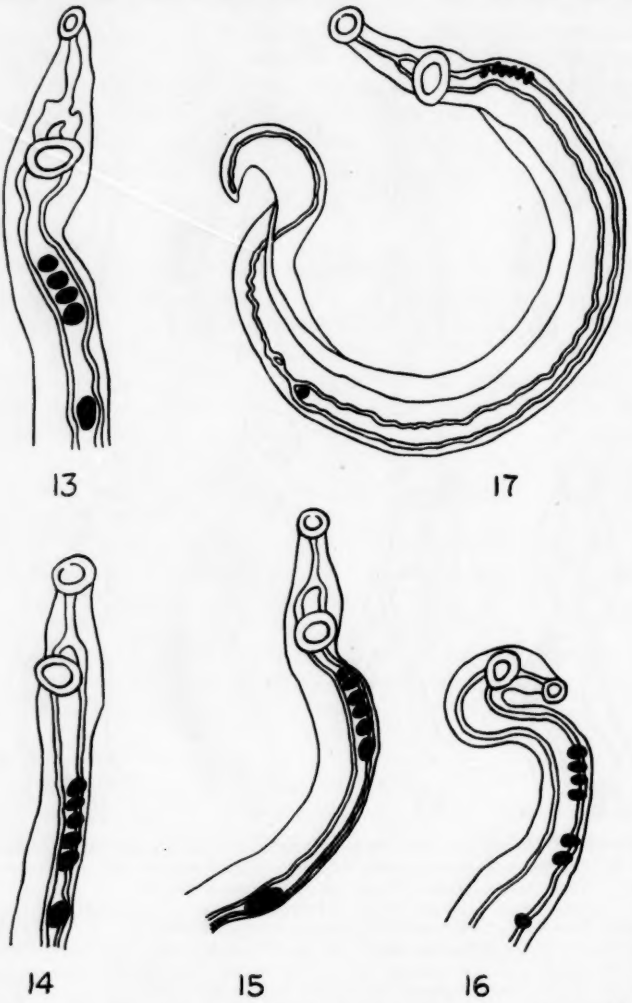
FIGURE 5–12. *S. japonicum*, male, showing specimens with various numbers of testes.
FIGURE 5. One testis, Japanese strain, 232 days, from a cat.
FIGURE 6. Three testes, Chinese strain, 60 days, from a mouse.
FIGURE 7. Four testes, Japanese strain, 123 days, from *Macacus cyclopis*.
FIGURE 8. Five testes, Formosan strain, 94 days, from a rabbit.
FIGURE 9. Six testes, Formosan strain, 64 days, from a mouse.
FIGURE 10. Eight testes, Formosan strain, 64 days, from a mouse.
FIGURE 11. Nine testes, Japanese strain, 232 days, from a cat.
FIGURE 12. Japanese strain, 112 days, from *Macacus cyclopis*.

the Formosan strain differed significantly in this characteristic from those of the Japanese.

(4) *Hermaphroditic Males.*

Among the 2,547 male specimens from the bi-sexually infected animals, 6 hermaphroditic ones were found. The over-all percentage is 0.2. All 6 specimens were

obtained from animals infected with the Chinese strain. The over-all percentage in the Chinese strain is 1.7, whereas it is zero in the other strains. Three specimens were obtained from a dog, 2 from 2 mice, and 1 from a hamster. The age of the worms from the dog was 87 days, from the mice 60–87 days, and from the hamster



FIGURES 13–17. *S. japonicum*, Formosan strain, male, 60 to 91 days, from rabbits, showing specimens with various arrangements of testes in unusual locations.

47 days. One specimen had 1 testis only; one had 3; one, 4; two, 7; and one had no record of the number of testes because the specimen was incomplete. As to female sexual organs, 4 specimens possessed only a rudimentary ovary; one specimen had a deformed ovary, and a blind-ended thin-walled empty uterus; and one had a

fairly well-developed ovary, and a uterus which contained a few deformed atrophied ova, but which showed no genital opening.

The guinea pig, hamster, and rabbit, animals known to be prone to the production of hermaphroditic specimens in male unisexual infections of *S. mansoni* (Vogel, 1947), were infected with male cercariae of the Japanese and Formosan strains of *S. japonicum*. Altogether 293 male specimens of the Formosan strain (69 from 3 guinea pigs and 224 from 10 hamsters) and 1,224 specimens of the Japanese (156 from 5 guinea pigs, 265 from 3 hamsters, and 803 from 4 rabbits) were obtained. The age of the worms varied from 57 to 74 days. No hermaphroditic specimens were found.

DISCUSSION

There has been much discussion concerning the effects of fixation on the arrangement of testes in trematodes. It is true that in *S. japonicum* these effects have to be taken into consideration. However, if the specimens are all alive when collected, and fixed under the same physical and chemical conditions, such effects, if any, will be on the same basis. The arrangement of testes in these specimens can therefore be used for comparison. Consequently, the significant percentage of specimens with non-tandem testes in *S. japonicum* must be regarded as a natural disposition of this parasite.

Seven has been regarded as a constant number for the testes in male *S. japonicum* (Katsurada, 1914; Faust and Meleney, 1924; Mao and Li, 1948). Up to this time only a few specimens with the number other than 7 have been reported (Vogel, 1947; Mao and Li, 1948; Moore, 1955). The present study shows that the number of testes in 10% of the male *S. japonicum* is not consistently 7. The number of testes next in frequency to 7 is 6. In this respect, the finding differs from that of Garcia (1953), who reported that 8 testes comes next to 7 in frequency.

What we have described as "testes in unusual locations" in this paper is what other investigators have designated as "supernumerary testes". The term "specimen with a supernumerary testis" should refer exclusively to one which possesses an extra number of testes in addition to the usual group of 7. As, in our specimens, isolated testis or testes can be added to the main group of 7, or other than 7, the term "testes in unusual locations" seems more suited to denote this situation than the term "supernumerary testes".

The so-called "supernumerary testes" in the genus *Schistosoma* were first reported by Vogel (1947) in *S. japonicum*. Moore (1955) reported the finding of 2 specimens with "supernumerary testes" for the Formosan strain of *S. japonicum* from 2 hamsters. It is interesting therefore to see that 36 more specimens with testes in unusual location are now added to the record for the Formosan, Japanese, and Chinese strains of *S. japonicum*. The location of a testis in front of the reunited portion of the intestinal ceca is also of great interest.

Hermaphroditic males have been repeatedly reported in *S. mansoni* from the guinea pig, hamster, rabbit, and mouse (Vogel, 1947; Short, 1948; LaGrange and Scheecqmans, 1949; Buttner, 1950). Vogel did not find a hermaphroditic male *S. japonicum* among the specimens in his possession. Moore (1955) reported the finding of one in a hamster infected with the Formosan strain. We are now adding to the record 6 more hermaphroditic males of the Chinese strain from the dog, hamster, and mouse. Three of them have a testicular number less than 7. In

the specimens which possessed only 3 testes, the outline and contents of the testicular structure were only faintly stained, simulating the suppressed testes in a hermaphroditic specimen given in Vogel's Figure 1,c. The fact that in hermaphroditic males the female organs may have developed at the expense of the male glands (Vogel, 1947; Short, 1951) is well-illustrated in our specimens.

The guinea pig, hamster, and rabbit has usually been regarded as favorable hosts for the finding of hermaphroditic males of *S. mansoni* (Vogel, 1947; LaGrange and Scheecqmans, 1944; Buttner, 1950), especially if the animals have been infected only with the male cercariae. It is of interest that in our animals, no hermaphroditic males were found in the guinea pig, hamster, and rabbit which had been infected only with male cercariae of the Formosan and Japanese strains of *S. japonicum*. *S. mansoni* seems more prone to the production of hermaphroditic males than *S. japonicum*.

The effects of the host-species and the parasite strains on the number and arrangement of the male sexual glands in *S. japonicum* have been well-illustrated. The predominance of the tandem or non-tandem testes is influenced by the specificity of the host. The arrangement of the testes also varies according to the strain. As to testes in unusual locations, there is also an interstrain variation, in addition to the host-species variations. For the number of testes, although no host-species variations are indicated, there are interstrain variations. As the Chinese strain distinguishes itself by the production of hermaphroditic males, the existence of interstrain variations in *S. japonicum* is also shown in hermaphroditism. If a parasitic species possesses several geographical strains, as in the case of *S. japonicum*, the characteristics of each strain must be thoroughly investigated. It is a fact that many of the so-called intra-specific variations of a parasite are closely related with the strain variations. The host-species is another important factor which influences the interstrain, intraspecific, as well as the interspecific, variations.

So far as the male sexual glands are concerned, each of the 4 strains of *S. japonicum* does differ from the others. There is no indication that the Chinese, Japanese, and Philippine strains, all anthropophilic, are always more closely related with one another than with the Formsan, the zoophilic strain. It seems to us that each strain has developed its own characters, although all 4 strains may have originated from 1 ancestral stem. One strain may have characteristics similar to 1 sister strain on the one hand, and others corresponding with another sister strain on the other hand.

SUMMARY

In total, 4,064 male *S. japonicum* were studied. They consisted of individuals from the Chinese, Formosan, Japanese, and Philippine strains, and were obtained from 13 animal host-species.

The arrangement of testes in *S. japonicum* can be divided into 2 classes, tandem, and non-tandem. The over-all percentage was 47.9 for the tandem group, and 52.1 for the non-tandem. The arrangement was influenced by 2 factors, the host-species, and the strain.

The number of testes varied from 1 to 14. The over-all percentages were 90.5 for the 7-testes, and 9.5 for other than 7-testes. Although the host-species variations of the number of testes in a strain were not significant, the strain variations

were significant among all the strains except the variations between the Chinese and Formosan.

Specimens with testis or testes in unusual locations have been found, and total 2.5%. Among the host-species, the rabbit infected with the Chinese strain gave the highest percentage, or 9.8. In the rabbit infected with the Japanese strain, the percentage was only 1.5. There is a strain variation in addition to the host-species variation.

Six hermaphroditic males were obtained from the animals bisexually infected with the Chinese strain, while none was found in the animals infected with the Formosan, Japanese, or Philippine. Experiments on the guinea pig, hamster, and rabbit, infected unisexually with male cercariae of the Formosan and Japanese strains, did not give hermaphroditic specimens.

The number and arrangement of the testes in *S. japonicum* show great intraspecific variations. There is no indication that the Chinese, Japanese, and Philippine strains, all anthropophilic, are always more closely related with one another than with the Formosan strain, the zoophilic. Each of the 4 strains of *S. japonicum* has developed its own characteristics.

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We wish to express our sincere appreciation to Dr. H. Vogel, Professor of Parasitology, Tropeninstitut, Hamburg, Germany, for his generous supply of the snail, *Oncomelania hupensis,* to Dr. Chang Chi-Kang, Director, National Institute of Health, Formosa, China, for his interest and help, and to Dr. Robert V. Hogg, Associate Professor, Department of Mathematics and Astronmy, State University of Iowa, for his advice in making statistical analysis of the data.

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A REDESCRIPTION OF *PSEUDODIORCHIS REYNOLDSI* (JONES, 1944) (CESTODA: HYMENOLEPIDIDAE), A PARASITE OF THE SHORT-TAILED SHREW

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Diorchis reynoldsi was described by Jones (1944) from the short-tailed shrew, *Blarina brevicauda*. This species held a unique position in the genus *Diorchis* since it was the only species reported from other than an avian host. In addition, the other completely known species in the genus possessed only 10 rostellar hooks, while *D. reynoldsi* possessed "about 100" minute hooks. In their work on the hymenolepidid cestodes of mammals, Skrjabin and Matevosian (1948) erected the genus *Pseudodiorchis* with *Pseudodiorchis reynoldsi* (Jones, 1944) as the type and only species. Schiller (1953), apparently unaware of the article of Skrjabin and Matevosian, reported that he had examined the type specimen of *Diorchis reynoldsi* and that there were 3 testes rather than 2 as reported in the original diagnosis. He recommended, therefore, that *D. reynoldsi* be assigned to the genus *Hymenolepis*. He substantiated this recommendation by noting that the rostellar armature of this species is more characteristic of the genus *Hymenolepis* than it is of *Diorchis*.

Pseudodiorchis reynoldsi has been recovered from 18 of 93 short-tailed shrews which have been examined from central Ohio since 1950. Most of these identifications have been based upon the armature of the rostellum since it is very difficult to obtain good material of this species. Recently, however, 47 mature and immature specimens of this species were obtained from a short-tailed shrew collected in Franklin County, Ohio. These specimens were alive when removed from the host; they were relaxed briefly in tap water and fixed in alcohol- acetic acid-formalin. The specimens were stained with Semichon's aceto-carmine, cleared in terpineol, and mounted in piccolyte. Only 10 mature strobilae were sufficiently relaxed to allow a detailed study of the internal anatomy.

It was soon apparent that the number of testes is variable in this species. In the majority of the proglottids, only 2 testes are present. Not infrequently, however, there are 3 testes, and rarely there may be 1 or 4 testes present. In addition, the examination of 4 squash mounts of scolices indicated that there are about 250 rostellar hooks rather than about 100 as originally reported.

An examination of the type specimen of *Pseudodiorchis reynoldsi* was made possible through the kindness of Mr. Allen McIntosh, Zoological Division, Agricultural Research Center, Beltsville, Maryland. It was impossible to compare my specimens closely with the type specimen because of the highly contracted condition of the latter, but from the shape, size, and distribution of the rostellar hooks, it seems highly probable that my specimens and the type specimen belong to the same species.

There can be no question of the close affinity of this cestode with the members of the genus *Hymenolepis*. However, evidence will be presented later which indicates that this worm is also related to the genus *Protogynella* Jones, 1943. There-

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fore the genus *Pseudodiorchis* proposed by Skrjabin and Matevosian may be of merit in indicating a transition from *Hymenolepis* to *Protogynella.*

Pseudodiorchis Skrjabin and Matevosian, 1948 genus emended

Diagnosis: Hymenolepidinae in which the lip of the terminal sucker of the rostellum is armed with a large number of minute hooks (approximately 250 in the type species) which are disposed in several closely overlapping rows. Testes variable in number; usually there are only 2 testes but occasionally there is a third testis which shows a retarded development. Genital pores unilateral; uterus sac-like. Parasites of shrews. Type species: *Pseudodiorchis reynoldsi* (Jones, 1944).

Pseudodiorchis reynoldsi (Jones, 1944) Skrjabin and Matevosian, 1948

Synonyms: Diorchis reynoldsi Jones, 1944;
Hymenolepis reynoldsi (Jones, 1944) Schiller, 1953

Diagnosis: (Measurements, unless otherwise stated, are in microns.) Total length 20–32 mm; greatest width, attained in gravid proglottids, 297–414. Scolex 345–450 in diameter, with 4 unarmed suckers 128–165 in diameter. Rostellum a muscular, protrusible organ 90–122 in diameter, surrounded by a rostellar sac 207–276 in diameter and 255–324 long. Rostellum armed with approximately 250 hooks disposed in several closely overlapping rows; hooks 4–5 long. Scolex followed by a short neck region and from 178–300 proglottids. In relaxed specimens, the proglottids are not well defined externally; in contracted specimens, proglottids are separated by slight constrictions. Mature proglottids 83–103 long and 186–248 wide; gravid proglottids 186–242 long and 297–414 wide. Genital pores unilateral and dextral. Excretory system composed of 2 pairs of longitudinal canals ventral to genital ducts; canals without transverse connectives; ventral canals 6–9 in diameter; dorsal canals about 2 in diameter. Testes variable in number; usually there are 2 testes transversely located in the posterior part of the proglottid; not infrequently there is a third testis located anterior and between the posterior testes. Posterior testes attain a maximum size of 34–48 in diameter in late immature and early mature proglottids; anterior testis, when present, usually smaller, 13–32 in diameter. Cirrus sac 68–82 long and about 17 in diameter, extending to the mid-point of the proglottid; cirrus spinose. Internal and external seminal vesicles present. Vagina ventral, anterior, or posterior to cirrus sac; the coiled condition of the vagina originally described for this species not apparent in the material studied. Seminal receptacle very prominent in fully mature proglottids. Ovary usually 3-lobed, taking up much of the space between the excretory canals. Vitellaria spheroid, 28–47 in diameter, located posterior and aporal to ovary. Uterus sac-like with incomplete dividing septa, taking up most of the space between the excretory canals. Eggs 56–62 long and 45–51 wide; 3 egg membranes present, inner membrane with 4 filaments at each pole. Larval hooks 12–13 long.

Host: Blarina b. brevicauda (Say).

Habitat: Intestine.

Distribution: Charlottesville, Virginia (type locality); Delaware, Franklin, and Hocking Counties, Ohio.

Specimens: In addition to the type specimen, a slide bearing a whole specimen from Franklin Co., Ohio, has been deposited in the Helminthological Collection of the U. S. National Museum, No. 55257.

Development and variation in the number of testes. The testes were counted in 465 late immature and early mature proglottids in 10 strobilae. These data are summarized in Table I. Considering the total number of proglottids observed, 61.1% contained 2 testes and 37.4% contained 3 testes. However, the incidence of proglottids with 2 and those with 3 testes varies considerably when each strobila is considered individually. For example, in strobila number 3, 91.8% of the proglottids had only 2 testes. In strobila number 2, on the other hand, 61.0% of the proglottids contained 3 testes.

When only 2 testes are present, they are usually located side by side in the posterior part of the proglottid. The third testis, when present, is usually located anterior and between the posterior testes. This anterior testis shows an unusual development which may explain in part the variation in testicular number which occurs in this species.

The testes are the first organs to develop. The posterior testes reach their maximum size in late immature proglottids before the female system becomes distinct; in these proglottids the posterior testes range from 34 to 48 (Ave. 40) microns in size. The anterior testis in these same proglottids, if present, is small and compact, ranging in size from 13 to 20 (Ave. 17) microns (Fig. 1). As the proglottid matures, the posterior testes become vesicular and gradually decrease in size as sperm are released (size range from 27 to 37 microns). The anterior testis becomes functional and somewhat larger in mature proglottids (size range from 17 to 32 microns), but it rarely reaches a size comparable to the maximum size reached by the posterior testes.

TABLE I.—*Variation in the number of testes in* Pseudodiorchis reynoldsi.

Strobila number	Number of proglottids observed	Per cent proglottids with number of testes indicated			
		1	2	3	4
1	59	3.4	49.2	47.4	0
2	59	1.7	35.6	61.0	1.7
3	61	1.6	91.8	6.6	0
4	32	0	46.9	50.0	3.1
5	60	1.7	83.3	15.0	0
6	71	0	53.3	46.5	0
7	38	0	63.2	37.8	0
8	14	0	57.2	42.8	0
9	43	0	67.4	32.6	0
10	28	0	50.0	50.0	0
Totals	465	1.1	61.1	37.4	0.4

DISCUSSION

The presence of 3 testes in 37% of the proglottids of *Pseudodiorchis reynoldsi* indicates the close relationship between this species and the species in the genus *Hymenolepis*. Within the genus *Hymenolepis*, variation in testicular number is not uncommon. Mayhew (1925) points out that in almost any species in the genus *Hymenolepis*, proglottids with 1, 2, or 4 testes are occasionally found. Voge (1952) studied the morphological variations in *Hymenolepis diminuta* (Rudolphi, 1819), *H. citelli* (McLeod, 1933), and *H. horrida* (von Linstow, 1901). In each of these species a small number of proglottids was found which contained from 1 to 6 testes. In *H. neosouthwelli* Hughes, 1940, the number of testes is not constant, many proglottids possessing only 2 (Southwell, 1922). However, in no species in the genus *Hymenolepis* which has come to the writer's attention does the reduction in testicular number approach the frequency of that found in *Pseudodiorchis reynoldsi*.

Hymenolepis diorchis Fuhrmann, 1913, is somewhat similar to *P. reynoldsi* since only 2 testes are well developed, the third being rudimentary. In this species, however, the rudimentary testis never becomes functional. Fuhrmann considered this species to illustrate clearly the transition from *Hymenolepis* to the 2-testicular genus *Diorchis*.

Mayhew (1925) suggested that each of the 3 testes of the species assigned to the genus *Hymenolepis* was formed phylogenetically by the fusion of at least 4 simple testes. As evidence to support this hypothesis, he noted irregularities in the number of vasa efferentia arising from single testes and also the lobing of testes as seen in some species. In a small number of proglottids which contained only 2 testes in *Hymenolepis sacciperium* Mayhew, 1925, he sometimes found groups of cells in the position of the missing testis which stained weakly in a manner similar to those

of reproductive cells. He concluded that the reduction in testicular number in these proglottids was caused by either a fusion of 2 testes or by the failure of one of the testes to develop. Voge (1952) also postulated that atrophy of testicular Anlagen in early segments may account for the lack of the usual number of testes.

The retarded development of the anterior testis in *Pseudodiorchis reynoldsi* together with the fact that it is missing in over half of the proglottids indicates that this species is evolving in the direction of a 2-testicular form. This reduction from 3 to 2 testes seems to be due to the fact that the anterior testicular primordium is unstable and frequently fails to develop. Whether this method of testicular reduction has been active in the evolution of the genera in the Hymenolepididae in contrast to Mayhew's hypothesis of testicular fusion is controversial. However, the evidence shown by *Pseudodiorchis reynoldsi, Hymenolepis diorchis,* and *H. sacciperium* tends to support the hypothesis that the reduction in testicular number in the genera of Hymenolepididae was caused by a gradual loss of testicular primordia rather than by a fusion of 2 or more primordia.

Jones (1943) erected the genus *Protogynella* for an aberrant hymenolepidid cestode from the short-tailed shrew. Oswald (1955) redescribed *P. blarinae* and provisionally added a second species to the genus. *Pseudodiorchis reynoldsi* and *Protogynella blarinae* show several striking similarities. Both species have been reported from *Blarina brevicauda* in the same geographical areas of Ohio and Virginia. According to Oswald (1955), the rostellum of *P. blarinae* is a muscular, protrusible organ armed with about 250 hooks which are arranged in 2 or 3 overlapping rows or tiers. The hooks are about 4 microns long. If it were not for the larger size of the scolex of *Pseudodiorchis reynoldsi,* it would be almost impossible to differentiate these 2 species on the basis of scolex characters. Another striking similarity is in the eggs of these 2 species, since each has prominent filaments at the poles of the inner egg membrane. In *Protogynella blarinae* there are only 2 filaments at each pole while the eggs of *Pseudodiorchis reynoldsi* are provided with 4 filaments at each pole.

Protogynella differs from *Pseudodiorchis* in that there is only 1 testis in each proglottid. In addition *Protogynella blarinae* is one of the smallest cestodes known; mature specimens of this species are less than one millimeter long. The similarities between *Pseudodiorchis reynoldsi* and *P. blarinae* may indicate that *Protogynella* was derived from a form very similar to *Pseudodiorchis* by the loss of 2 of the ancestral 3 testes. The fact that 1 testis of *P. reynoldsi* is unstable lends credence to this hypothesis.

SUMMARY

Pseudodiorchis reynoldsi (Jones, 1944) is redescribed from specimens obtained from *Blarina brevicauda* in Ohio. The number of testes in this cestode is variable; 61.1% of 465 proglottids which were examined contained only 2 testes while 37.4% contained 3 testes. One testis appears to be unstable and is either missing or shows a retarded development.

This species is compared with *Protogynella blarinae,* and the reduction in the number of testes in the Hymenolepididae is discussed.

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PLATE I

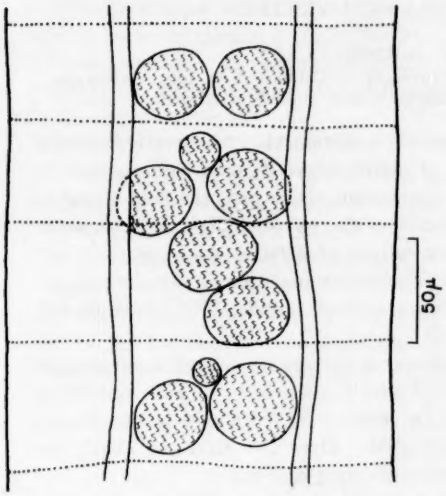

FIG. 1

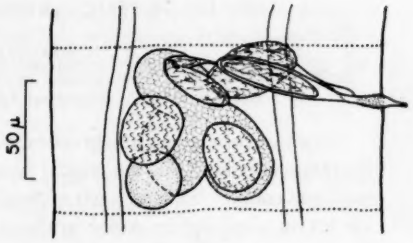

FIG. 2

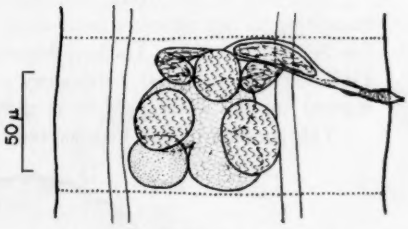

FIG. 3

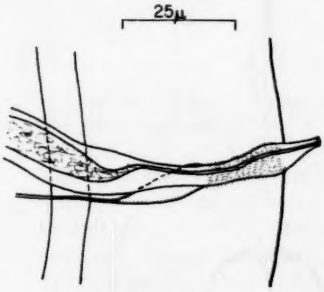

FIG. 4

FIG. 5

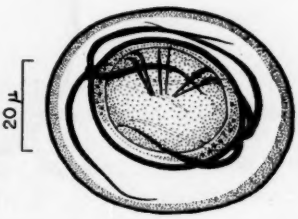

FIG. 6

EXPLANATION OF PLATE

Pseudodiorchis reynoldsi (Jones, 1944)

FIGURE 1. Four consecutive immature proglottids showing variation in the number of testes.
FIGURE 2. Mature proglottid with 2 testes, dorsal view.
FIGURE 3. Mature proglottid with 3 testes, dorsal view.
FIGURE 4. Cirrus and vagina, dorsal view.
FIGURE 5. Rostellar hooks.
FIGURE 6. Egg.

THE COPEPOD *CHONIOSPHAERA CANCRORUM* PARASITIZING A NEW HOST, THE GREEN CRAB *CARCINIDES MAENAS**

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Scripps Institution of Oceanography of the University of California, La Jolla, California, and The Marine Biological Laboratory, Woods Hole, Massachusetts

During recent years, the green crab, *Carcinides maenas* (L.) has greatly extended its range northward in the coastal waters of Maine where its enormous increase in numbers coupled with its habit of feeding upon young clams has been held responsible for an alarming reduction in the population of the soft-shell clam *Mya arenaria*. For this reason *Carcinides* has become the subject of detailed study by the Clam Investigations of the U. S. Fish and Wildlife Service in order to elucidate all aspects of its biology that may contribute to its control (Glude, 1955; Scattergood, 1952; Dow and Wallace, 1952).

As a part of this study, samples of green crab containing an unknown parasite inhabiting its egg masses were sent by Mr. Walter R. Welch, U. S. Fish and Wildlife Service, Boothbay Harbor, Maine, to Dr. Horace W. Stunkard at the Woods Hole Marine Biological Laboratory in July 1956. Here Dr. Stunkard kindly entrusted samples of the parasite to me for identification and study.

This parasite (Fig. 1) turned out to be a species of copepod which was described

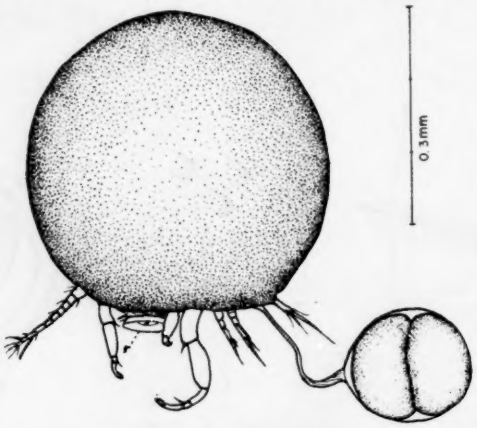

FIGURE 1. *Choniosphaera cancrorum*. Adult female with eggs. r, rostrum.

by C. J. Connolly in 1929 as a new genus and species, *Choniosphaera cancrorum* of the family Choniostomatidae. This family of crustacean parasites has received but a smattering of study on the American coasts. Aside from Connolly's paper the only other reports appear to be those of Blake (1929) in which 3 new species of the genus *Sphaeronella* are described, and of Paul Illg (in MacGinitie 1955) recording several specimens of *Choniostoma mirabile* Hansen from the shrimp *Eualus gaimardi* off Point Barrow, Alaska.

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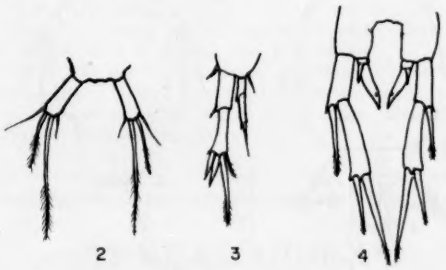
* Contribution from Scripps Institution of Oceanography New Series No. 928, and the Woods Hole Marine Biological Laboratory.

As the specific name indicates, Connolly found his species on the eggs of cancer crabs, *Cancer amoenus* Herbst, *Cancer irroratus* Say, and *Cancer borealis* Stimpson. He reports the species from St. Andrews, New Brunswick, and from Woods Hole, Massachusetts. Thus the specimens from Boothbay Harbor, Maine, fall well within the distributional range of the species.

Collections from the marsupium of the green crab

The finding of this same species of parasite on the eggs of the green crab, which belongs to a different family, is of some biological interest in view of Hansen's (1897) observation that several of the same species of Choniostomatidae may live on 2 or more host species of the same genus or of 2 closely related genera and "perhaps in exceptional cases they may be found on animals of different families."

The present specimens do not completely agree with the original descriptions given for *Choniosphaera cancrorum*. For example the caudal rami of the adult female, with 1 segment each (Fig. 2), are typical of copepods generally. Connolly



FIGURES 2, 3 and 4. *Choniosphaera cancrorum*. Adult female. (2.) caudal rami; (3.) first leg; (4.) second pair of legs.

states that the rami are 2-jointed. His figures and descriptions of the thoracic feet are incomplete but he infers that only the exopods are composed of 2 segments though his figures suggest also 2-segmented endopods. In the present material, the endopods possess 2 segments, a short first segment and a second longer segment (Figs. 3 and 4). Other minor differences occur in the spination of the thoracic legs.

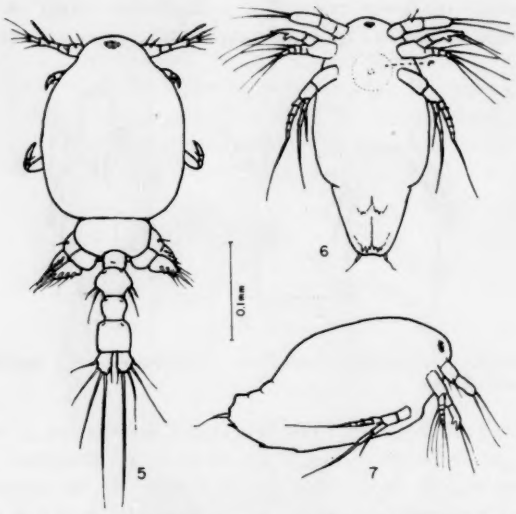
In his "cyclops" stage he finds only 1 spine on the posterior lateral angles of the first urosome segment. In my specimens there are 2 spines as shown in Figure 5. It seems likely that all of these differences represent oversights or misinterpretations.

The adult females (Fig. 1) are spherical or subspherical in shape, range in size from 0.35 mm to 0.42 mm in length (evidently depending upon spawning condition) and rather closely resemble the eggs of the host crab in size and color. They were observed to be able to move freely about amongst the eggs and in this respect differ from the description which Wilson (1932, p. 493) gives for members of the Lernaeopodoida which includes the family Choniostomatidae. He says: "The female becomes a fixed parasite attached immovably to its host." In other respects *Choniosphaera cancrorum* appears to be less degenerate than other members of the suborder and also of the family Choniostomatidae (See Hansen 1897, 1923).

The adult female lays her eggs in small packets of 1 to 4 eggs each. These packets are attached to the genital area by a long slender cord. Many egg packets

were also found scattered throughout the crab egg-mass where they are attached by shorter cords to the stalks of the crab eggs or to hairs of the swimmerets. A nauplius larva hatches from the egg.

In general appearance the nauplius is much like that of free-living copepods but differs greatly in details and, as already observed by Connolly, no well defined stages could be determined in its development (Figs. 6 and 7). The bases of the second antennae are not provided with masticatory hooks and the penultimate segment of the endopods bear 2 terminal setae (Connolly shows but 1) and a small lateral process or spur. A distinctive character of the larvae, but one difficult to observe in most specimens, is the presence of the funnel-like "rostrum" which is situated between the larval mandibular palps. This structure becomes a striking feature in the "cyclops" and adult stages when the mouth and mandibular blades can be observed within the membranous circle.



FIGURES 5, 6 and 7. *Choniosphaera cancrorum*. (5.) copepodid ("cyclops") stage; (6.) late nauplius stage, ventral; (7.) late nauplius stage, lateral. r, rostrum.

In addition to the adult female, numerous free-living nauplii and copepodid ("cyclops") stages were found in the marsupium of the crab. The male was not found.

The copepodid stage (Fig. 5) which metaporphoses from the nauplius is very active and probably represents the principal stage which spreads infection from host to host, though unattached eggs may also float into the water to hatch in the vicinity of uninfected crabs.

Glude (1955) introduces briefly the idea of green crab control through the agency of natural enemies. Such biological control might eventually stabilize the crab population in the new range to a level that will enable clam recruitment sufficient to support a moderate clam fishery. The effect of the present parasite as a natural control is not known and warrants further study in order to determine, if possible, whether or not the *Choniosphaera* attack on the green crab is indeed an

adaptation coincident with the extension of *Carcinides* range. In the present study it has been possible to examine only a few crabs. Connolly's (1929) observations that the parasite occurs in the Woods Hole region on *Cancer amoenus* would lead one to believe that green crabs in that area might also be infected, but he makes no mention of having examined other crabs than *Cancer*.

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RE-DESCRIPTION OF *IXODES LUNATUS* NEUMANN 1907 AND OF A NEW SPECIES CONFUSED THEREWITH, FROM MADAGASCAR

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Much confusion exists concerning the true diagnosis of *Ixodes lunatus* Neumann 1907, a species which is known to be endemic in Madagascar. This species was originally described from *Hallomys audeberti* Jentinck and the type female is deposited in the Natural History Museum of Leiden. Other females which agree substantially with the type have been collected from *Centites ecaudatus* from Madagascar (No. 75.7.20.22, British Museum (Natural History) collections) and 1 female from *Setifer setosus*, RTB 9830, Kilo 68, Tamatave Road, Madagascar, 4 March 1952, V. J. Lipton *legit* (private collection of the writer). Recently, however, Colas-Belcour & Grenier (1942) have published a redescription of a female, and have described the male and the larva of ticks which they consider to be *I. lunatus* Neumann 1907. Their description fits more closely specimens of a new species *I. colas-belcouri* collected from *Rattus rattus* in Madagascar by Lt. Tipton of the Army Medical Service Graduate School and which Hoogstraal (1953) also named *Ixodes lunatus*. Through the courtesy of Mr. Harry Hoogstraal I have been able to re-examine these specimens. All measurements cited in the text are in mm.

Ixodes lunatus Neumann 1907
(Figures 1–5)

Redescription of female

All specimens well engorged, unfed type of Neumann measures 2.7×1.8; body broadest towards the posterior third, narrowing appreciably anteriorly; alloscutum with hairs of moderate length. Color brown.

Capitulum (Figs. 1, 2): Length of basis 0.66–0.68, width of basis behind palpal insertion 0.63–0.65. Basis capituli triangular, distance between cheliceral foramen and palpal insertion greater than distance between the insertion and dorsal ridge. Dorsal ridge sinuous and terminating posterolaterally in broadly rounded cornua, whose basal breadths are greater than their lengths. Lateral margins slightly curved, divergent proximally, becoming indented opposite article I, thence straight and convergent to the cheliceral foramen. Surface flat: more heavily pigmented along the margins. Porose areas depressed, elongate oval, long axis slightly oblique to long axis of body (cf. Neumann 1907, Figs. 1, 2). Palpi long, about 5 times as long as the greatest breadth, lateral profile almost straight except for a basolateral undulation, inner margin of article II convex, with greatest width at about its distal quarter; inner margin of article III straight, apex rounded being more sharply curved nearer the midline. Length of article II, 0.62–0.63, article III, 0.30–0.32, greatest width of 0.18–0.19. Palpal hairs short on article III except near the apex (Fig. 1), those on article II longer on inner margin than on outer margin. Rostral apex conspicuously ridged behind cheliceral foramen. Ventrally basis capituli long, broad, attaining greatest breadth across auriculae, latter as long pointed retrograde spurs, directed postero-ventrally (thus appearing to be foreshortened in Fig. 2); posterior margin almost straight, postero-lateral angles broadly rounded, constriction slight. Flap-like spur on article I, inner surface of article II flat, that of article III slightly concave.

Hypostome: missing in all specimens examined.

Scutum (Fig. 3): Length 1.66–1.67–1.69, greatest breadth of 1.22–1.24–1.25 at about mid-length; narrow elliptical, maroon-brown, glossy, glabrous. Cervical grooves shallow, narrow, extending back for most of the scutal length, not reaching postero-lateral border. Lateral carinae lacking, but scutum downcurved laterally. Scapulae short, pointed, emargination moderate. Punctations small, shallow, uniformly distributed and well separated from one another.

Legs: Moderate in length and thickness. Coxa I with broad external spur, internal spur of about the same length, tapering to a long sharp point, external spurs on coxae II and III of

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similar shape to that on I but shorter; external spur on coxa IV short, broad and rounded apically. Trochanters lacking spurs (Fig. 4). Tarsus I tapering gradually from proximal end to Haller's organ (Fig. 5) with a slight hump in front of the latter, tarsus IV of similar form but shorter and relatively broader, length of tarsus I, 0.85, metatarsus I, 0.49; tarsus IV, 0.64, metatarsus IV, 0.52.

Anal groove: Horseshoe-shaped.

Vulva: Between posterior edges of coxae III.

Spiracular plate: Located nearer to the vulva than to the anus. Subcircular, macula antero-ventral. Goblets small, numerous.

This description and the figures agree substantially with those of Neumann (1907). The only major disagreements concern the form of the palps which Neumann described and figured as narrow and cultriform but which we find have straight outer margins and convex inner margins. Neumann gives the location of the vulva between coxae IV; we find it is more anterior than this. The description given by Colas-Belcour & Grenier (1942) however, contrasts strongly with that of Neumann and that presented herewith, and is discussed later.

Ixodes colas-belcouri new species
(Figures 13–37)

Holotype: Female collected from *Rattus rattus alexandrinus*, Ambatofinandrahana, Madagascar, 29 Jan. 1952, V. J. Tipton *legit.* RTB 9808. Deposited in the U. S. National Museum.

Allotype: Male collected from *Rattus rattus alexandrinus*, RTB 9809, Ambatofinandrahana, Madagascar, 20 Jan. 1952, V. J. Tipton *legit.* Deposited in the U. S. National Museum.

Paratypes: Total 4 females, 1 male, 3 nymphs; 2 females, 1 male, 1 nymph with same data as holotype and allotype; 1 female, *Tenrec ecaudatus*, RTB 9811 Ambohimahosa, Madagascar, 6 Feb. 1952, V. J. Tipton *legit;* 1 nymph, *Rattus rattus*, RTB 9820, 60 Kilo on Tamatave Road, Madagascar, 29 Feb. 1952, V. J. Tipton *legit;* 3 females, 1 nymph, *Rattus rattus*, RTB 9805, Tanandavy, Madagascar, 30 Jan. 1952, V. J. Tipton *legit; 2* larvae, shrew, RTB 9822, 69 Kilo on Tamatave Road, Madagascar, 29 Feb. 1952, V. J. Tipton *legit.* Paratypes are deposited in H. Hoogstraal collection, NAMRU-3, Cairo, the Rocky Mountain Laboratory, Montana, the British Museum (Natural History) and the author's private collection.

Description of female

Compared with *Ixodes lunatus* Neumann the present new species is small (Figs. 1, 13; 2, 14; 4, 16, all of which are drawn to the same scale), overall dimensions of a slightly replete female being 1.85×0.82 and of more fully engorged specimens, 2.3×1.2. Body broadly rounded behind, narrowing appreciably anteriorly.

Capitulum (Figs. 13, 14, 15): Basis capituli triangular with its greater length lying in front of the palpal insertion; broader than long, greatest width 0.32–0.33–0.34, length from dorsal ridge to cheliceral foramen, 0.27–0.29–0.31. Dorsal ridge sinuous, terminating postero-laterally in pointed cornua, which are as long or longer than their basal widths. Lateral margins behind palpal insertion short, curved, thence almost straight to cheliceral foramen. Surface of basis elevated near the margin (lined in Fig. 13) being particularly so adjacent to the porose areas, surface convex between these lateral elevations. Porose areas transversely oval in most specimens, with long axis transverse to that of the body, but in some specimens (e.g. RTB 9805) these areas are superficial, round, consisting of very few, widely scattered pores. Palpi nearly 6 times as long as broad; length of article II, 0.25–0.26–0.28, length of article III, 0.18–0.21–0.22; greatest width, 0.08–0.09. Outer margin of article II undulate basally, then almost straight to the apex of article III, inner margin convex with greatest width proximally at base of article III, apex of latter much as in *I. lunatus*. In ventral view basis is relatively narrow, attaining greatest width across auriculae. Auriculae as sharply pointed retrograde spurs which are produced into distinct points antero-laterally (Figs. 2, 14). Posterior margin almost straight, postero-lateral angles curved; sides slightly constricted just behind the auriculae. Two pairs of hairs in position indicated in Figure 14. Flap-like spur on article I; inner surface of articles II and III flat. Palpal hairs few and short.

Hypostome (Fig. 15): Length 0.315, broadest proximally and narrowing to an apical point. Denticles large laterally and arranged as 4 rows of 4/4, 4 rows of 3/3, 3 rows of 2/2, behind an apical corona.

Scutum (Fig. 17): Length 0.87–0.91–0.93, greatest width of 0.67–0.69–0.72 at or behind mid-length; sides curved and terminating in a broadly rounded posterior edge. Scapulae short, pointed, emargination distinct. Lateral carinae prominent, long but not reaching postero-lateral

margins, inner slopes as short, steep ridges, with moderate slopes laterally. Cervical grooves as shallow depressions, convergent at first, then divergent and extending back to just behind the posterior limits of the lateral carinae. Punctations small, fairly widely dispersed and uniformly distributed. Hairs fine, of moderate length and fairly numerous but shorter than those on the alloscutum where they are stouter and curved.

Legs (Figs. 16, 18a, b): Moderate in length and size. Coxae I–III with extensive syncoxal areas (shaded in Fig. 16), coxa I with long, sharp, narrow internal spur, external spur shorter and broader; coxae II and III lacking both spurs, external spur on coxa IV pointed and as long as that on coxa I or slightly longer. Tarsus I with a short sharp hump subapically, but otherwise almost subparallel to Haller's organ, tarsus IV tapers more strongly from the proximal to the distal end. Length of tarsus I, 0.43, metatarsus I, 0.24; tarsus IV, 0.4, metatarsus IV, 0.25. Trochanters with strong, pointed, postero-external spurs. Hairs short and small.

Genital orifice: Placed on a level with the third intercoxal space.

Anal aperture: Placed very near posterior limit of body, anal groove elliptical, and rather broad.

Spiracular plate: Circular, macula anterior to center. Goblets large, not numerous. Dimensions in holotype, 0.2×0.2.

Description of male

Very small, elongate oval, dimensions in allotype 1.5×0.77. Legs long with last 2 segments of legs IV extending back beyond posterior limits of body. Color of body pale brown, capitulum more heavily pigmented.

Capitulum (Figs. 20, 21, 22): Base color brown, with a paler band extending continuously from about halfway along the sides and the posterior edge (limits indicated by a fine line in Fig. 20). Dorsal ridge straight, salient. Cornua short and pointed but nevertheless distinct. Lateral borders quite strongly convex before converging opposite the palpal insertions. Ventrolateral margins declivitous. Surface convex in the middle and separated from strongly elevated longitudinal ridges (heavy line in Fig. 20) by broad deep depressions (stippled in Fig. 20); shiny with reticulate markings and few scattered punctations. Palpi short, twice as long as the greatest breadth; length of article II, 0.12, length of article III, 0.10, suture line between these articles ill-defined. Article III tapers gradually from the suture line to the apex; outer margin of article II undulate, inner margin strongly and broadly convex, and defines the greatest width of the palp. Article I overlapped dorsally by an antero-lateral extension of the basis capituli. Palpal bases widely separated. In ventral view the posterior edge of the basis concave, sides also concave and divergent to insertion of palps. Transverse ridge strongly defined and consisting of a large,median, pointed, triangular eminence and 2 smaller pointed projections in the position of auriculae; steep surface from the transverse ridge to the hypostomal base, surface behind ridge elevated laterally into triangular-shaped cuticular prominences, and depressed in the middle. The anterior and inner face of the coxae lie close to the sides of the basis (heavy lines in Fig. 21).

Hypostome (Fig. 22): Length 0.09, breadth across basal teeth, 0.075. Apically indented. Denticles arranged in 6 files of lateral teeth; proximally there is a very long postero-ventrally directed basal tooth with a smaller and more sharply angled tooth (sub-basal tooth) at its base; distal to the long tooth the dentition is 1 row of 1/1, 1 row of 2/2, 1 row of 3/3, 1 row of 2½/2½, whilst towards the midline and subtending the long tooth (basal tooth) there is a single crenulation.

Scutum: Elongate oval, nearly twice as long as broad, length 1.2, breadth 0.65. Sides straight for most of their length, but converge quite strongly at the level of second intercoxal space; posterior margin broadly rounded. Scapulae pointed, emargination moderate; surface with small, uniformly distributed punctations, hairs very fine, short; hairs on marginal body fold longer and coarser. Lateral carinae lacking. Cervical grooves ill defined, shallow and extending to about one-third scutal length; triangular depressions lateral to the ends of cervical grooves (stippled in Fig. 25).

Ventral plates (Fig. 26): Pregenital plate extends from level of second to that of the first intercoxal space, posterior margin straight, lateral margins slightly convergent, anterior edge rounded in one specimen, indented in the allotype, as figured. Median plate slightly longer than broad, and reaches from near anterior third of coxae III to 1/3 the distance from coxae IV to the posterior margin. Anterior margin slightly concave, antero-lateral borders long, postero-lateral borders short, posterior margin straight. Adanal plates broadest anteriorly, mesially concave, laterally convex. Anal plate horseshoe-shaped. All plates finely punctuate bearing hairs which are longer than those on the scutum. As in a number of other African males of this genus there is a piriform plate mesial to coxa I and on the intersegmental membrane a number of chitinous plaques, some of which bear short hairs.

Legs (Figs. 24, 26): Moderate length and thickness; last 2 segments of legs IV extend beyond posterior edge of body (Fig. 25). Coxae I–III with rather extensive syncoxal areas.

Coxa I with minute, but nevertheless distinct, marginal salience. Coxa II almost square, coxae III broader than long due to the extreme posterior extension of the synocoxae which overlap the anterior edge of coxae IV; coxae IV large, subtriangular with prominent tapering external spur. Spurs lacking on coxae II and III. Distinct, pointed trochantal spurs on legs III and IV. Tarsi relatively long, tarsus I sharply humped distal to Haller's organ, tarsus IV without a hump; length of tarsus I, 0.39, metatarsus I, 0.18; tarsus IV, 0.34, metatarsus IV, 0.21.

Spiracular plate (Fig. 23): Elongate oval, long axis corresponds to that of the body, macula fairly central. Goblets large, not numerous. Length 0.175, breadth 0.12.

Description of nymph

All specimens examined engorged and measure about 1.2×0.7. Body small, suboval.

Capitulum (Fig. 27): Length of basis from dorsal ridge to apex of cheliceral foramen, 0.155, breadth across dorsal ridge, 0.21. In dorsal view basis triangular in shape with sinuous dorsal ridge drawn out to strong points laterally, lateral margins converging from the postero-lateral extremities, profile sinuous. Surface flat, reticulately patterned. Palpi club-like, broad distally and narrowing quite strongly proximally, suture line between articles II and III faint; combined lengths of articles II and III, 0.20. In ventral view basis broad, particularly across the auriculae; the latter are bidentate, consisting of a sharp laterally directed point and a broader, less strongly pointed postero-laterally directed spur (Fig. 28). Basis behind the auriculae broadly curved and strongly constricted.

Scutum (Fig. 29): Broadly oval, narrowing more strongly in front, length 0.42, breadth across the widest part at about mid-length, 0.45. Scapulae short, rounded. Lateral carinae and cervical grooves lacking. Surface flat except antero-laterally where there is a perceptible downwardly curving slope; smooth, finely punctuate, bearing a few fine white hairs.

Legs: Of moderate length and breadth. Coxa I with small internal spur and a broader external spur of similar length, distinct external spurs on all other coxae, but lacking internal spurs (Fig. 31). Tarsi short, broad; tarsus I with short sharp hump, length of tarsus I, 0.25, length of metatarsus I, 0.12 (Fig. 30).

Spiracular plate: Transversely oval.

Description of larva

Very small, suboval in outline.

Capitulum (Fig. 32): Basis much as in the nymph; length from dorsal ridge to hypostomal apex, 0.19, breadth across dorsal ridge, 0.16. In ventral view posterior and postero-lateral margins broadly rounded, constriction very slight. Auriculae large blunt. Palpi long, more than 5 times as long as broad; combined length of articles II and III, 0.16; swelling distally with a latero-basal indentation on article II. Palpal hairs short.

Hypostome: Length 0.095. Pointed apically, teeth long, arranged in about 12 rows of 2/2 files, behind a slight corona, median triangular unarmed area.

Scutum (Fig. 37): Polygonal, angles rounded.

Legs (Figs. 35, 36): Relatively short, broad. Coxa I with external spur slightly longer than internal spur; coxae II and III with slightly salient external spurs, which are very weak on coxa III. Tarsi relatively short and broad; tarsus I with Haller's organ about mid-length, claw as long as the prominent pulvillus.

Related species: *Ixodes lunatus* Neumann is a larger species than *Ixodes cola-belcouri* n. sp. These species can be separated from one another on coxal armature, *I. lunatus* having external spurs on all coxae, which decrease in size from coxae I to IV, whereas in *I. colas-belcouri* external spurs are lacking in coxae II and III, whilst those on coxae IV are as long or longer than the corresponding spurs on coxae I. Differences are also apparent in the form of the porose areas (Figs. 1 and 13). The auriculae of *I. colas-belcouri* have sharp anterior projections which are wanting in *I. lunatus*.

In considering these related species attention must be directed to the ticks described as *I. lunatus* by Colas-Belcour & Grenier (1942). I have been unable to see the original specimens described by these authors, but from the data presented by them I am of the opinion that the specimens they describe are not *I. lunatus* in sensu Neumann 1907. For comparison with *I. colas-belcouri* a free translation of Colas-Belcour & Grenier's description of the male and female of the ticks they consider to be *I. lunatus* is given, together with the drawings taken from their paper (Figs. 6–12).

Female. The capitulum in the female specimen is mutilated. The female measured 1.7 mm long (without capitulum); maximum breadth, 1.3 mm. Basis capituli triangular with slight cornua (Fig. 7—not clear from the figure). Porose areas transversely oval, interval between

them considerably less than their length. Auriculae are long, pointed, retrograde spurs, longer than the internal spur on coxa I. Scutum 1.57 × 1.39 mm (otherwise similar to that given by Neumann). Postero-internally to coxa I are 2 small sclerotized plaques. Vaginal orifice on a level with coxae IV, genital grooves markedly divergent. Coxae I with 2 short squat spurs separated by a straight posterior border (Fig. 6); an accessory tuberosity on the postero-internal angle and anterior to the internal spur. Postero-internal marginal saliences on coxae II and III. No external spurs (other than slight projections) on coxae II and III. External spur on coxae IV strong, tapering, pointed, longer than that on coxa I. Coxae I to III syncoxae. Trochanters II, III and IV bear strong pointed spurs at their distal extremities.

Male. Length of body without capitulum, 1.2 mm, greatest width 0.75 mm. Shape sub-oval, narrower in front than behind (Fig. 10); red-brown color. Hypostome short, broad; indented slightly at the apex, where the corona consists of 1 small tooth on each side, 4 transverse rows of teeth arranged in 3 rows of 2/2 teeth and a basal row of 2/2 (Fig. 9). The lower part bears 2 large lateral teeth, subtended basally by 2 smaller teeth, within the angle of the large lateral teeth are 2 angled projections (= basal row of hypostomes described in other African males). Chelicerae long, strongly armed; dorsal process terminates in a recurved hook-like tooth; internal article with several teeth of which the two inferior ones are the strongest. Palps rounded, spatulate. Article I reduced, directed outwards and forwards, article II longer than broad. Dorsal ridge of basis almost straight, terminating postero-laterally in sharply angled cornua. Ventrally the transverse ridge bears a large median triangular projection, with smaller triangular projections (Fig. 10—"deux bosses arrondies") in the position of the auriculae. Scutum oval, regular, body fold quite broad; furnished with fine punctuations which are uniformly distributed, and without hairs. Grooves not visible. Ventrally and mesial to the inner edge of coxa I and 2 piriform sclerotizations. Pregenital plate well developed, extending from the first intercoxal space to the second intercoxal space; a median indentation anteriorly. Posteriorly terminates in a genital apron overlying the vaginal orifice at the level of the second intercoxal space. Median plate very large, reaches from near anterior third of coxae III to 1/3 of the distance from coxae IV to the posterior margin of the body; of irregular pentagonal form, twice as long as broad, anterior margin concave (corresponding to the level of the genital orifice), lateral margins more or less straight, posterior margin much broader than anterior margin; limited by the edge of the adanal plates, bears a number of hairs. Adanal plates comma-shaped, greatest width anteriorly, mesial borders concave where they adjoin anal plate. Latter bounded by circular anal groove, open behind. Anus, limited by chitinous "ring," closed by 2 valves supplied with 2 pairs of median hairs and pair of posterior hairs on the "ring." The anal plate is crescentic and chitinous. A line of large sclerotized granulations, some with hairs, extend from the small piriform plates anteriorly to the posterior border. Coxae I–III syncoxae. Postero-internal and internal angles of coxa I sharp but insufficiently so to be called true spurs. Coxae II and III unarmed, coxa IV with large external spur; trochanters II, III and IV with spurs on their distal extremities. Spiracular plate elongate oval, reddish brown color, epimeral plates much reduced. Except for certain insignificant details, our description of the larva agrees substantially with that of Colas-Belcour & Grenier (1942).

Comparison of these data with the present findings leads me to believe that Colas-Belcour & Grenier (*loc. cit*) were either describing *Ixodes colas-belcouri* n. sp. or else a very closely allied form. Such differences as do exist may be due to the fact that the material of Colas-Belcour and Grenier was mounted in Berlese's fluid whereas my material was unmounted. The major differences in our descriptions are in the form of the basis capituli (Figs. 7 and 13 and 6 and 14) of the female, in the presence of coxal saliences other than spurs in Colas-Belcour and Grenier's specimens of the female and the male; differences in the form of the hypostome (cf. Figs. 9 and 22), coxal shape and partly in the dimensions of the ventral plates of the male (cf. Figs. 10 and 26). Such differences may well be due to distortion as a result of mounting, but until I have seen the material on which Colas-Belocur and Grenier based their descriptions I hesitate to make a final statement on this point. On the other hand I state, without hesitation, that these authors are in error in describing their specimens as *I. lunatus.*

Remarks: I name this species for Dr. J. Colas-Belcour of the Institut Pasteur in recognition of his contributions to our knowledge of various parasitological problems, not the least being the ticks.

Hosts: Rats, *Rattus rattus alexandrinus, Rattus rattus* subsp.?, *Tenrec ecaudatus.*

Distribution: Known only from Madagascar at present.

SUMMARY

1. The female of *Ixodes lunatus* Neumann 1907 is re-described from new material collected in Madagascar and its host range is now extended to include *Centites ecaudatus* and *Setifer setosus.*

2. *Ixodes colas-belcouri* new species is described from females, males, nymphs and larvae collected from rats and *Tenrec ecaudatus* at Ambohimahosa, Tamatave Road and Tanandavy, Madagascar. This new species has been confused with *Ixodes lunatus* but is readily separated from the latter by the absence of external spurs on coxae II and III, a very long external spur on coxae IV, and also in the shape of the auriculae.

3. The species assigned to *I. lunatus* by Colas-Belcour & Grenier (1942) is in all probability *I. colas-belcouri.*

ACKNOWLEDGMENTS

I am very grateful to Mr. Glen M. Kohls for reading the manuscript and for his helpful comments.

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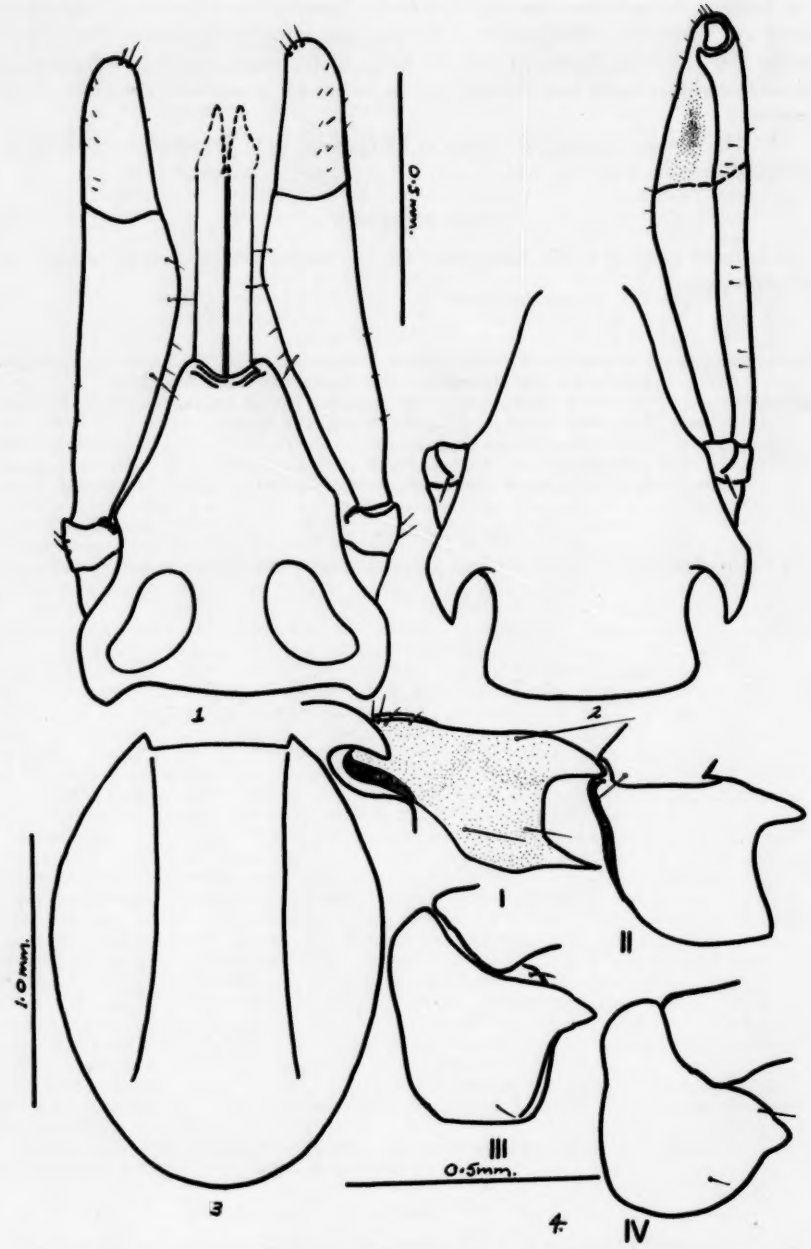
EXPLANATION OF FIGURES

With the exception of Figures 6–12, all comparable figures are drawn to the same scale.

PLATE I



FIGURES 1–4. *Ixodes lunatus* female: 1. Capitulum, dorsal; 2. Capitulum, ventral; 3. Scutum; 4. Coxae I–IV.

PLATE II

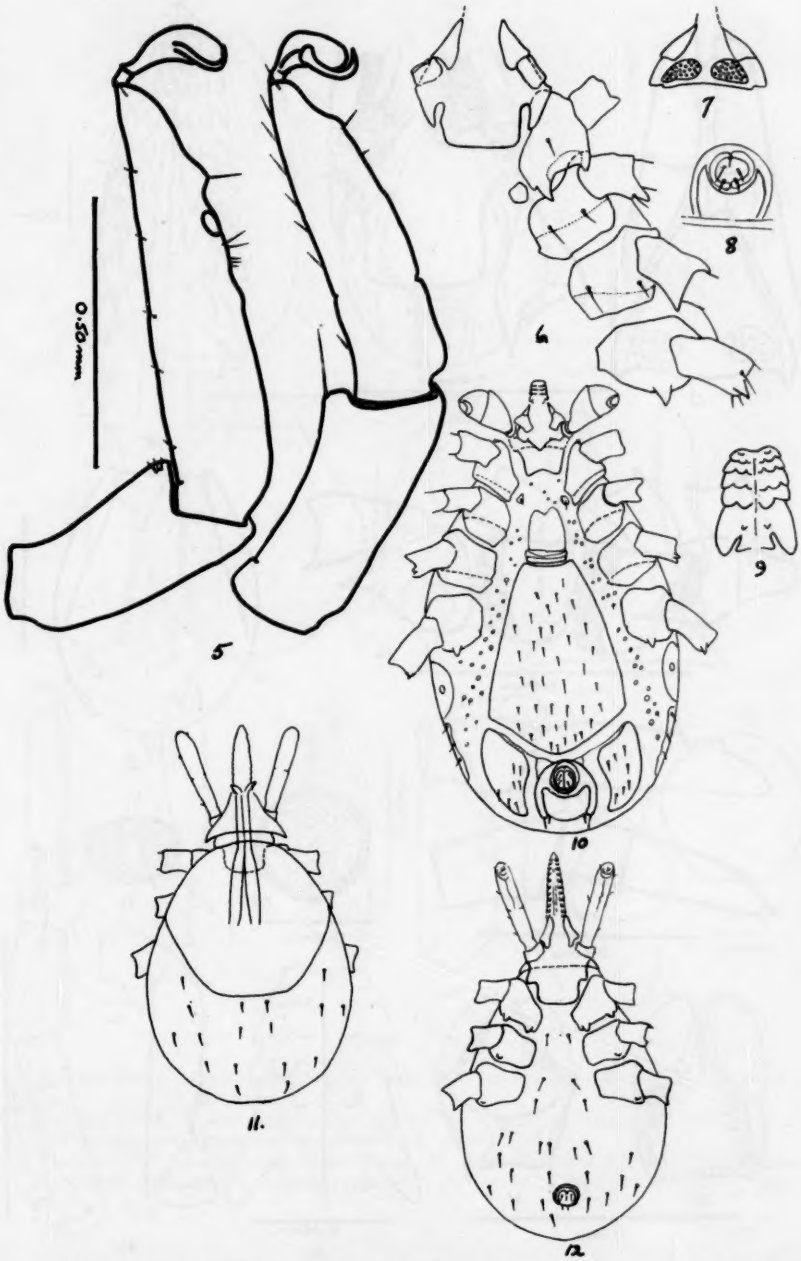
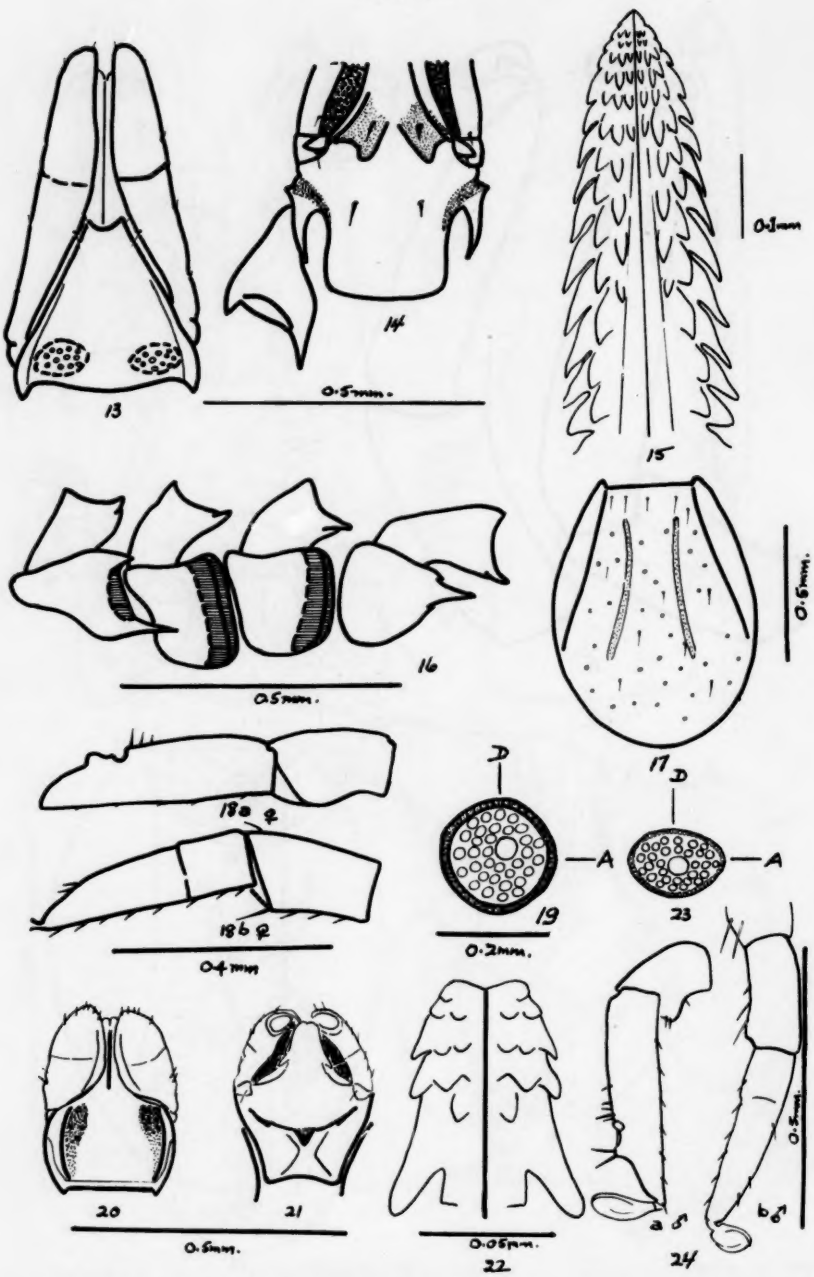


FIGURE 5. *Ixodes lunatus* female: a. Tarsus and metatarsus I; b. Tarsus and metatarsus IV.
FIGURES 6–11. Redrawn from Colas-Belcour and Grenier (1942) and described by them as *I. lunatus* (see text): 6. Basis capituli and coxae I–IV; 7. Basis capituli, dorsal; 8. Anal plate; 9. Hypostome of male; 10. Venter of male; 11. Dorsum of larva; 12. Venter of larva.

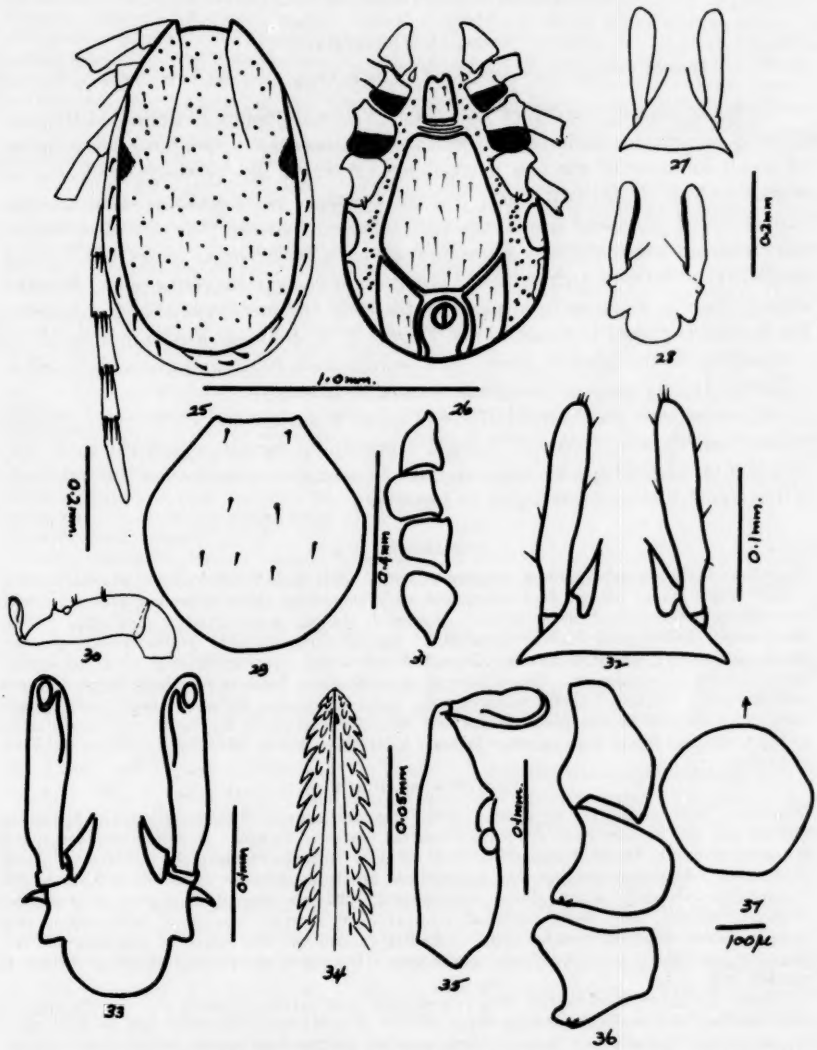
PLATE III



FIGURES 13–19. *Ixodes colas-belcouri* female: 13. Capitulum, dorsal; 14. Capitulum and coxa I, ventral; 15. Hypostome; 16. Coxae and trochanters I–IV; 17. Scutum; 18a. Tarsus and metatarsus I; 18b. Tarsus and metatarsus IV; 19. Spiracle.

FIGURES 20–24. *Ixodes colas-belcouri* male: 20. Capitulum, dorsal; 21. Capitulum, ventral; 22. Hypostome; 23. Spiracular plate; 24a. Tarsus and metatarsus I; 24b. Tarsus and metatarsus IV.

PLATE IV



FIGURES 25, 26. *Ixodes colas-belcouri* male: 25. Opisthosoma, dorsal; 26. Opisthosoma, ventral.

FIGURES 27–31. *Ixodes colas-belcouri* nymph: 27. Capitulum, dorsal; 28. Capitulum, ventral; 29. Scutum; 30. Tarsus and metatarsus I; 31. Coxae I–IV.

FIGURES 32–37. *Ixodes colas-belcouri* larva: 32. Capitulum, dorsal; 33. Capitulum, ventral; 34. Hypostome (drawn from mounted specimen); 35. Tarsus I; 36. Coxae I–III; 37. Scutum.

CESTRAHELMINS LARUEI N.G., N. SP., A DIGENETIC TREMATODE FROM THE MUSKELLUNGE, *ESOX M. MASQUINONGY* MITCHILL*

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During a parasite survey of northwest Wisconsin fishes Fischthal (1950) recorded a moderate infection (12 worms) of *Macroderoides spiniferus* occurring in the small intestine of the one muskellunge, *Esox m. masquinongy,* collected on August 1, 1945 from Teal Lake, Sawyer County. A re-study of this material through serial cross and frontal sections indicated that the previous identification was erroneous and that a new genus and species, *Cestrahelmins laruei,* of uncertain familial relationship is represented. The generic name (Gr. *cestra,* dart + *helmins,* worm) refers to the numerous spines on the body and in the cirrus and metraterm. The species is named in honor of Dr. George R. LaRue, my former professor and a consultant in the present study. All worms were fixed in Lavdowsky's AFA, stained in Harris' hematoxylin, and mounted in balsam.

Appreciation is due Allen McIntosh, George R. LaRue and E. W. Price, Animal Disease and Parasite Research Branch, Agricultural Research Service, U. S. Department of Agriculture, for examining whole mounts and sections of the trematode of this report, and commenting on its identity.

Cestrahelmins n. g.

Diagnosis: Body elongate, spinose, swollen at neck. Oral sucker subterminal; prepharynx extremely short, almost non-existent; esophagus very long; ceca short, extending half the length of hindbody. Acetabulum pre-equatorial. Testes 2, oblique, postovarian in hindbody. Cirrus pouch large, extending far back of acetabulum; contains large bipartite seminal vesicle, prostate glands, and spined, eversible cirrus. Genital atrium small, opening sinistral, close to antero-lateral margin of acetabulum. Ovary dextral, pretesticular. Seminal receptacle large. Laurer's canal present. Vitelline follicles postacetabular and pretesticular, in lateral fields meeting dorsally. Uterine coils occupy posttesticular and testicular regions of hindbody; metraterm large, saccular, covered inside with acicular spines. Excretory bladder saccular. Parasite of freshwater fish.

Cestrahelmins laruei n. sp.

Diagnosis: With characters of genus. Body elongate, spinose, swollen at neck. Spines on anterior end and swollen neck fine; much coarser posterior to neck; spination becomes sparse in region of ovary, finally disappearing from all body surfaces immediately posterior to testes. Oral sucker subterminal, smaller than acetabulum; latter just anterior to middle of body length. Prepharynx extremely short, almost non-existent; pharynx muscular, slightly oval-shaped; esophagus slender, very long; intestinal bifurcation at anterior margin of acetabulum; ceca slender, medial to lateral vitelline fields, extending caudad half the length of hindbody and terminating just behind posterior limits of vitellaria. Remnants of cercarial eyespots present in swollen neck.

Testes 2, approximately equal, relatively smooth, postovarian, obliquely placed, with anterior testis sinistral and posterior dextral, frequently in contact; vasa efferentia join to form short vas deferens. Cirrus pouch large, curved, anterior portion overlapping acetabulum considerably, extending well posterior to latter and terminating ventral to ovary or seminal receptacle. Seminal vesicle internal, bipartite, outermost chamber thin-walled, saccular, sperm-filled, approximately 3.4× longer than inner; latter thick-walled, not inflated with sperm. Prostate gland cells numerous around innermost chamber of seminal vesicle. Cirrus long, muscular, bearing long spines, inverted, protrusible, dextral to metraterm. Cirrus joins metraterm to form small genital atrium. Genital pore ventral, sinistral, close to antero-lateral margin of acetabulum.

Ovary dextral, postacetabular and pretesticular, intercecal, dorsal to posterior portion of cirrus pouch, relatively smooth. Oviduct short. Seminal receptacle large, sperm-filled; located

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* Contribution No. 10 from the Department of Biological Sciences.

between ovary and testes, overlapping all three. Laurer's canal present. Mehlis' gland well-developed, surrounding oötype. Uterus completely fills posttesticular and to a lesser extent testicular regions of hindbody; ascends ventral to ovary and cirrus pouch without coiling to enter postero-medial portion of metraterm; portion of ascending uterus near metraterm appears to have longitudinal rod-like structures in wall with same translucency as body spines. Metraterm sinistral to cirrus, large, saccular, lined with prominent cells whose free ends are rounded and bear fine, short, acicular spines. Vitelline follicles extracecal in lateral fields; extend from behind level of cirrus and metraterm to level of anterior testis; usually meet in narrow band dorsally. Eggs numerous, small, operculate with very slight collar and transparent shell; contains zygote surrounded by vitelline material.

Excretory pore terminal; bladder saccular, extending forward medially between uterine coils and testes to receive primary tubules anterior to posterior testis and opposite anterior testis.

Mean measurements in millimeters (with minima and maxima in parentheses) of 10 whole mount adult specimens are: body, length 1.446 (1.267–1.570), width (in ovarian region) 0.277 (0.251–0.330); oral sucker, 0.072 (0.066–0.075) × 0.077 (0.062–0.090); acetabulum, 0.121 (0.114–0.126) × 0.121 (0.108–0.128); prepharynx, length (in 1 much flattened specimen), 0.013; pharynx, 0.052 (0.048–0.053) × 0.044 (0.041–0.050); esophagus, length 0.385 (0.310–0.429); ovary, 0.120 (0.084–0.147) × 0.102 (0.083–0.120); metraterm, 0.121 (0.113–0.128) × 0.097 (0.090–0.113); eggs, 0.028 (0.023–0.031) × 0.014 (0.013–0.015); anterior testis, 0.120 (0.108–0.135) × 0.123 (0.092–0.165); posterior testis, 0.123 (0.099–0.142) × 0.120 (0.093–0.142); cirrus, 0.150 (0.143–0.157) × 0.063 (0.060–0.065); outermost chamber of seminal vesicle, 0.254 (0.210–0.297) × 0.106 (0.095–0.117); innermost chamber of seminal vesicle, 0.075 (0.063–0.110) × 0.071 (0.065–0.086); longitudinal extent of curved cirrus pouch, 0.390 (0.350–0.442); longitudinal extent of vitelline field, 0.298 (0.231–0.350); posterior margin of acetabulum to posterior end of body, 0.802 (0.607–0.910); posterior margin of vitelline field to posterior end of body, 0.434 (0.330–0.561); posterior margin of posterior testis to posterior end of body, 0.330 (0.231–0.376).

Host: Esox masquinongy masquinongy Mitchill.

Habitat: Small intestine.

Locality: Teal Lake, Sawyer County, Wisconsin, U.S.A.

Type: U.S. Nat. Mus. Helm. Coll., No. 55234 (1 slide of the type specimen, and 1 slide of another specimen in serial cross section).

In attempting to establish the identity of the parasite of this report Yamaguti's (1953) key to the families of the order Prosostomata from fishes was followed to the Monorchiidae. However, the worms could not be fitted into any existing genus. All specimens were sent to Allen McIntosh for aid in placing them. In a personal communication he stated, "Your slides . . . have been examined by Dr. LaRue, Dr. Price, and myself and none of us can recall having seen anything like it previously. We agree with you that it will almost fit into a variety of groups but differ in some anatomical features." A personal communication from Dr. LaRue stated, "Unfortunately your species seems to have a complex of characters which does not clearly place it in any of the recognized genera or families. For some reason it does not seem to fit the Monorchiidae, nor does it seem to fit the Fellodistomatidae to which it keyed out in one of the keys we used. In some respects it seems to fit the Lepocreadiidae better than anywhere else. The medium size of the egg makes it difficult to put in among the Plagiorchioidea. There are some members of this superfamily infecting fishes. But again the fit did not seem to be very good." He further stated that if more were known concerning the character of the excretory bladder, the worm might be more readily placed; the Lepocreadiidae and Monorchiidae probably form the bladder from a mass of mesodermal cells, but the Fellodistomatidae, at least the Gymnophallinae, retain the primitive form. Unfortunately sections of the adult worms do not show the method of formation of the bladder. Since life history stages are not available, it is not possible to settle either the character of the bladder or the affinities of this worm.

Cestrahelmins differs from the Fellodistomatidae Odhner, 1911, emended Nicoll, 1935, as characterized by Dawes (1946, 1947) and Yamaguti (1953) in that (1)

the cirrus pouch is long, extending far behind the acetabulum instead of short, never extending behind the acetabulum; (2) a seminal receptacle is present, not absent; (3) a metraterm is present, not absent; (4) the excretory bladder is saccular instead of Y- or V-shaped; and (5) the eggs are not embryonated when laid instead of being embryonated.

Cestrahelmins differs from the Monorchiidae Odhner, 1911, in that (1) the oral region has few cutaneous glands instead of numerous ones; (2) the esophagus is very long, not short; (3) the seminal receptacle is very large instead of very small or absent; (4) the seminal vesicle is of definite bipartite form, not saccular, and (5) the uterine coils are mainly posttesticular, not extending anterior to the testes, instead of coiling anterior to the 1 or 2 testes present.

According to LaRue (mimeographed data sheet) it seems to be impossible to retain the lepocreadiids with their peculiar trichocercous cercariae as a subfamily of the Allocreadiidae. He states that more life histories and a further analysis of genera are needed to establish the family Lepocreadiidae Nicoll, 1934, (superfamily Allocreadioidea) on a sound basis. *Cestrahelmins* differs from the Lepocreadiidae in that (1) the prepharynx is extremely short, almost non-existent, instead of well marked; and (2) the uterine coils are mainly posttesticular instead of pretesticular. Other differences may exist, but these will not be apparent until the family is more clearly defined.

In the superfamily Plagiorchioidea Dollfus, 1930, McMullen (1937) listed a number of families which parasitize fishes at least in part. These are Plagiorchiidae Lühe, 1901, Lissorchiidae Poche, 1926, Lecithodendriidae, Odhner, 1910, and Macroderoididae n. fam. *Cestrahelmins* differs from all these families in having a saccular bladder, whereas it is Y-shaped in the first 2 families, V-shaped in the third, and I-shaped in the fourth. Further, it differs from the Lissorchiidae in having (1) a cirrus pouch, not lacking one; and (2) the testes oblique in position instead of tandem. It differs further from the Macroderoididae in having (1) a spined cirrus and metraterm, not unspined; (2) a sinistral genital pore, not median; and (3) intestinal ceca which terminate at the middle of the hindbody instead of extending to the posterior end.

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Abbreviations

A, acetabulum; C, cirrus; CP, cirrus pouch; E, egg; EB, excretory bladder; GA, genital atrium; GP, genital pore; IC, intestinal cecum; M, metraterm; OD, oviduct; OV, ovary; P, parenchyma; PG, prostate glands; S, spine; SR, seminal receptacle; SVI, seminal vesicle, innermost chamber; SVO, seminal vesicle, outermost chamber; TA, anterior testis; TP, posterior testis; U, uterus; V, vitellaria.

Figure 1. Adult worm, ventral view.

Figure 2. Cross section through region of intestinal bifurcation.

Figure 3. Cross section through genital pore.

Figure 4. Cross section at point of junction of cirrus and metraterm.

Figure 5. Cross section through anterior portion of cirrus pouch and the metraterm.

Figure 6. Cross section through posterior portion of cirrus pouch.

Figure 7. Cross section through seminal vesicle, ovary and oviduct.

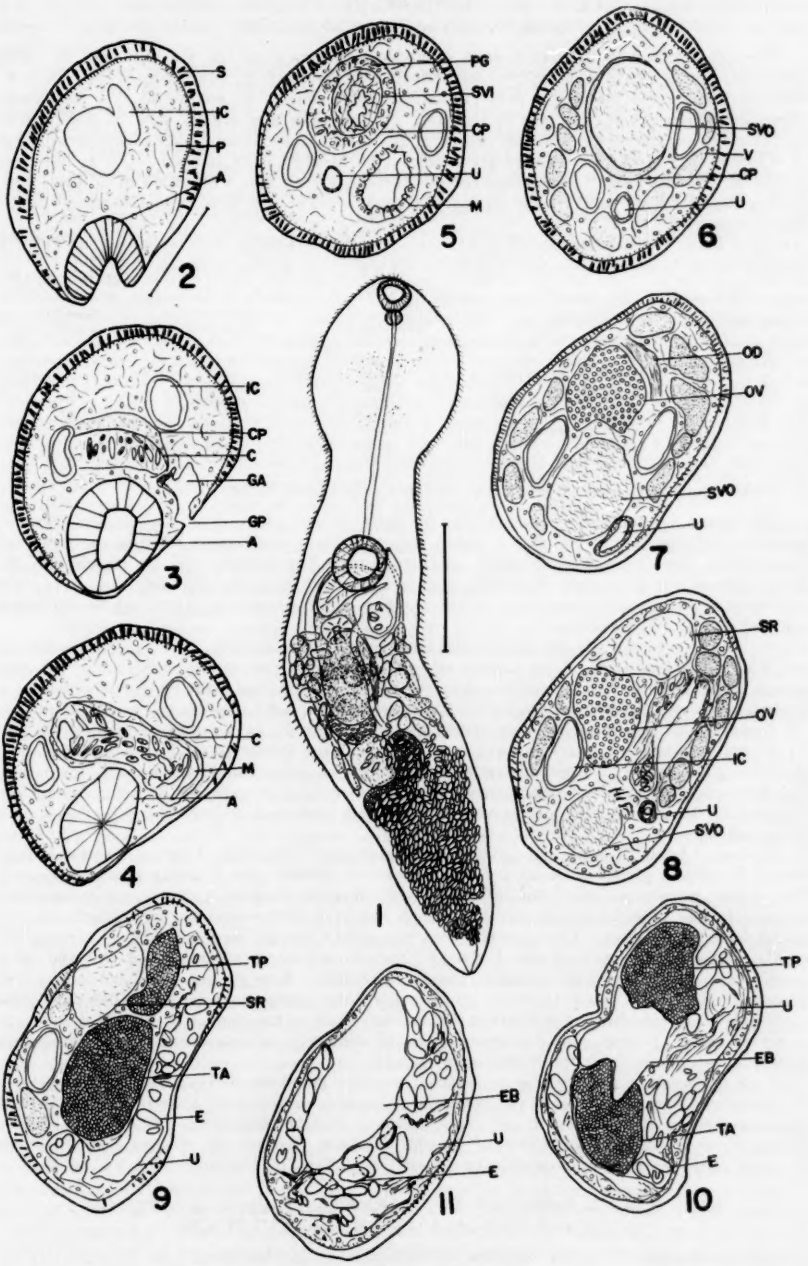
Figure 8. Cross section through seminal vesicle, ovary and seminal receptacle.

Figure 9. Cross section through seminal receptacle and testes.

Figure 10. Cross section through testes and excretory bladder.

Figure 11. Cross section through excretory bladder and uterus in posttesticular region.

PLATE I



EXPLANATION OF PLATE

All figures refer to *Cestrahelmins laruei,* and were drawn with the aid of a microprojector. The value of the scale is 0.3 mm for Figure 1, and 0.1 mm for Figure 2; the latter scale also applies to Figures 3–11.

RESEARCH NOTES

EXTENDED LOW-TEMPERATURE PRESERVATION OF HUMAN MALARIA PARASITES

The preservation in the frozen state of viable blood parasites for 404 days and of viable sporozoites for 375 days was reported by Jeffery and Rendtorff in 1955 (Exper. Parasitol. **4**: 445–454). Since this method of preservation has been adopted as routine at this laboratory, extension of these periods can now be reported. The methods used are the same as described in the prior report. Since the previous report, a total of 19 transmissions with blood parasites of all 4 species of *Plasmodium* of man preserved for 60 to 1,093 days at about −70° C has been successful. Of this total, 9 were preserved in excess of 1 year, and 3 in excess of 2 years. Six of the remainder were preserved in excess of 6 months. The others were stored for 60 to 121 days before use.

In an effort to determine the effect of these extended periods of preservation on the relative viability of the blood samples, paired samples are presented in the following table:

Pair No.	Species	Days of Preservation	Millions of Parasites	Days Prepatent Period
1.	*P. falciparum* (McLendon strain)	33*	85.7*	6*
		398	85.7	8
2.	*P. falciparum* (McLendon strain)	329	679.6	6
		739	679.6	6
3.	*P. falciparum* (Panama strain)	158*	57.2*	9*
		404*	57.2*	10*
4.	*P. ovale* (Donaldson strain)	399	156.6	13
		997	104.4	9

* These cases were reported previously (Jeffery and Rendtorff, 1955).

The donor for each paired sample was the same; it was intended to use identical amounts in each inoculation, but in Number 4, 1/3 of the sample was inadvertently lost in the 2nd trial. Nevertheless, this recipient experienced a shorter prepatent period than did the recipient of the whole amount. It is thought that these data indicate that little viability was lost during the preservation periods used, since the variations which did exist may be explained by individual susceptibility of the recipients.

In several instances frozen blood containing malaria parasites has been shipped packed in solid CO_2 to various parts of the country for use in treatment of neurosyphilitics. In the only reports which have been received concerning the viability of this blood the recipients experienced incubation periods of 12, 15, and 18 days, which were not unusually long for the species concerned (*P. vivax*). The specimens had been preserved for 240 and 272 days prior to shipment.

In only a single instance has there been an unqualified failure to produce a malaria infection through the use of frozen infected blood. This was not a paired sample, and the failure may have resulted from the initial freezing rather than from storage for an extended period. In addition, the susceptibility of the recipient was not confirmed by reinoculation with known viable parasites.

Two of 3 trials of preserved sporozoite samples were successful. One was a sample containing 20 salivary glands of *Anopheles quadrimaculatus* infected with *P. vivax* (Chesson strain). This sample, maintained frozen for 785 days, produced in the recipient a 16-day prepatent period identical to that required for an identical sample reported in the previous publication and preserved for only 375 days. The second success was with a sample containing 12 salivary glands of *A. quadrimaculatus* infected with *P. vivax* (Chesson strain); this sample had been preserved for 958 days and produced an incubation period of 14 days. A single uncomplicated failure was experienced with a *P. vivax* (Chesson strain) sporozoite sample preserved for 1044 days. No sample identical with this one had been tested earlier; so its failure cannot be attributed to length of preservation. In this case the susceptibility of the recipient was confirmed by subsequent successful inoculation with the homologous parasite.

Low temperature preservation of malaria parasites continues to be a satisfactory method. Preservation for at least 2 years appears to be reasonably certain with little if any loss in viability.—GEOFFREY M. JEFFERY, *U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Tropical Diseases, Post Office Box 717, Columbia, South Carolina.*

THE ESTABLISHMENT OF VARIOUS TRICHOMONADS OF ANIMALS AND MAN IN AXENIC CULTURES

The advantages of axenic cultures of trichomonads for biological and physiological investigations motivated studies to devise methods of establishing such cultures from infective

material obtained from chickens, turkeys, domestic pigeons, mourning doves, and Canada geese, and also from rats, swine, cattle, and man. Details of the medium and techniques employed are given here, together with information on the writer's experience with the establishment of such cultures.

The medium now used has evolved from the writer's experience with the C.P.L.M. medium of Johnson and Trussell (1943, Proc. Soc. Exper. Biol. and Med. **54**: 245–249) and its more important modifications: STS medium of Kupferberg, Johnson, and Sprince (1948, Proc. Soc. Exper. Biol. and Med. **67**: 304–308) and the medium of McEntegart (1952, J. Clin. Path. **5**: 275–280). The medium consists of trypticase (BBL), 2.0 g; yeast extract, 1.0 g; maltose, 0.5 g; L-cysteine hydrochloride, 0.1 g; L ascorbic acid, 0.02 g; agar, 0.05 g; distilled water to make 90 ml; sheep serum (inactivated, 56°C, 30 min.), 10 ml; potassium penicillin G, 100,000 units; and streptomycin sulfate, 0.1 g. The dry ingredients, exclusive of the antibiotics and agar, are first dissolved in the water, and the pH of the solution is adjusted to 6.8–7.0 with sodium hydroxide, or to pH 6.0 with hydrochloric acid, if *Trichomonas vaginalis* is to be cultured. The agar is then added and the mixture autoclaved for 10 minutes at 15 pounds pressure, 121°C. Upon cooling to 48°C the serum and antibiotics (the latter contained in 1.0 ml, distilled water) are added, and 5.0-ml quantities of the medium transferred, aseptically, into sterile, rubber-stoppered tubes (13×100 mm). Tubes so prepared may be stored at 4°C up to 14 days, possibly longer, without deterioration of the contained medium. Prior to use, they should be incubated at 35.5°C for 1 hour. Originally, the antibiotics were added to the tubes just before inoculation. After repeated trials had demonstrated that the potency of the antibiotics used did not decrease below an effective level when added to the medium at the time of preparation, they were routinely incorporated in the medium as described above. Experience has shown, however, that after the culture has been purified, antibiotics may be omitted. The medium as prepared with 0.05% agar concentration was employed for V-tube migration and stock maintenance of cultures; for isolation purposes 0.1% agar was employed.

Preparation of inocula was as follows: Vaginal material from various sources was obtained by aspiration with a glass pipette and rubber suction bulb. A small quantity of saline was first introduced into the region of the posterior fornix to thin the secretions for aspiration. Up to 1 ml of aspirated material was added per tube of medium. Nasal material was best obtained by washing the nasal passages with saline introduced under mild pressure with a rubber syringe. The washings were then centrifuged (1500 rpm, 10 min.), and up to 0.5 ml of sediment was inoculated per tube. Fecal or cecal material was emulsified in saline to such a consistency that it could be handled with a 1-ml serological pipette. One-half ml of emulsion sufficed as an inoculum. To obtain *Trichomonas gallinae* from the upper digestive tract of the avian host, the writer employed with excellent results a technique devised by C. M. Herman, U. S. Fish and Wildlife Service (unpublished). For this procedure a cotton swab was first prepared by dipping it into a solution of cellulose acetate in amyl alcohol and allowing it to dry. This treatment aided in preventing the entrapment of the flagellates between the cotton fibers. In use the dry swab was first introduced into the oral passages of the bird and brushed over the surfaces of the mucosa particularly beneath the tongue. It was then withdrawn, inserted in the upper layer of a tube of medium, and agitated to free the flagellates. After this the swab was reintroduced into the bird and a swab sample of the crop mucosa obtained. The same tube of medium received both samples.

The technique employed for the isolation and purification of cultures was essentially that described by Fitzgerald *et al* (1954, Cornell Vet. **44**: 302–310) for the culture of swine trichomonads. Tubes of isolation medium inoculated by overlaying the medium with the infected material were incubated for 48–96 hours at 35.5°C. The presence of trichomonads was determined by examining microscopically 0.01-ml samples of culture fluid taken at levels approximately halfway down and at the bottom of the tube.

It was possible on occasion to purify the cultures by repeated subculture through medium containing antibiotics. However, a higher percentage of successful results was obtained if the trichomonads were transferred from the isolation tube directly to a V-tube and allowed to migrate through it. The V-tubes employed were of Pyrex glass, bent so that an angle of about 35° separated the two arms, which were 120 mm in length and had an inside diameter of 13 mm. Each tube was filled with enough medium to produce a column of fluid approximately 75 ml in depth. To inoculate a tube, 0.5 ml of culture material was taken from the isolation tube and layered on top of the medium in one arm, and the tube incubated for 48–96 hours. Tests for the presence of trichomonads in the arm opposite to that inoculated were carried out by removing a 0.01-ml sample of culture at a level halfway down the column of fluid and examining it microscopically. As soon as trichomonads were found in a test sample, 0.5 ml of culture was withdrawn from the level examined for the organisms, and transferred to a tube of stock maintenance medium. After 96 hours of incubation in this medium, tests for the presence of con-

taminants were made in the following manner: Samples (0.02–0.05 ml) of the cultures were transferred to antibiotic-free medium for a period of 48–72 hours. From this medium subcultures (with 0.02 ml inoculum) were made to Fluid Thioglycollate Medium (BBL) and Brain Heart Infusion Medium (Difco) prepared with 0.1% agar, and incubated at both room temperature and 35.5°C for 7 days. Absence of growth at the end of this period was taken as evidence that the cultures were free of contaminants.

A single passage through a V-tube was ordinarily sufficient to purify a culture. However, on occasion it was necessary to repeat the operation. Once the culture was purified, it was maintained as stock culture by subculturing to fresh medium at intervals of 48 to 72 hours.

In this way axenic cultures of the following trichomonads have been established and maintained: One strain each of *Trichomonas gallinarum* from the chicken and turkey; 1 strain of a *T. gallinarum*-like form from the Canada goose (*Branta canadensis*); 1 strain of *T. eberthi* from the turkey; 2 strains each of *T. gallinae* from the domestic pigeon and mourning dove; 6 strains of the *T. foetus*-like trichomonad from the nasal passages of swine and a *T. batrachorum*-type from the swine cecum (Figs. 1 and 12 of Buttrey, 1956, J. Protozool. **3**: 8–13); 1 strain of *T. foetus* from the preputial space of a bull; 3 strains of a *T. batrachorum*-type from calf feces; 2 strains of *T. hominis* from man, and a single strain from a wild rat (*Rattus norwegicus*); and a single strain of *T. vaginalis* from a case of human vaginitis. The only trichomonad which the writer has failed to cultivate axenically by this method is *T. tenax* from the human mouth.

To the writer's knowledge this is the first report of the axenic culture of *T. eberthi*. Studies of stained culture material of the *T. batrachorum*-type of trichomonad isolated from calf feces indicate that the parasite is different from 2 previously described species from the gastrointestinal tract of cattle, *T. ruminantium* Braune 1914 and *T. enteris* Christl, 1954.

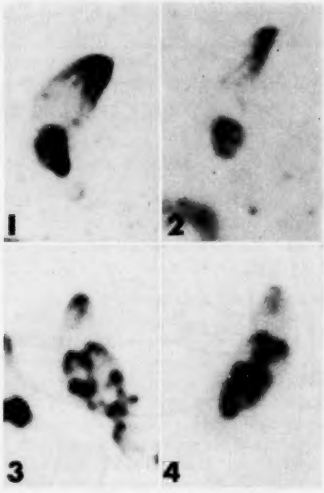
Incidental observations on the axenic cultures of trichomonads have revealed a number of interesting points: (1) Axenic cultures are easier to establish by employing trichomonads taken directly from the host than from mixed bacterial cultures which have been transferred repeatedly. (2) At first isolation, small numbers of parasites can successfully establish cultures. However, for succeeding axenic subcultures many more organisms are required in the inoculum to result in growth. This may be due to a lack of proper oxidation-reduction conditions in the medium, a factor compensated for in the original isolation tube by other microorganisms. From 50,000 to 200,000 organisms, depending on the species, are required to maintain good stock cultures. (3) It is possible, by maintaining axenic cultures at room temperatures which do not go below 25°C for 5 days and further incubation at about 35.5°C for 2 more days, to eliminate the need for more than 1 transfer of culture per week. To maintain stock cultures under this regimen approximately twice the number of organisms required above are necessary. (4) All of the trichomonads except *T. gallinarum* and the *T. gallinarum*-like trichomonads grow well at 35.5°C; the latter grow better at 38.5°C. (5) Methylene blue in the quantity employed in C.P.L.M. medium (0.0002%) inhibits growth of the *T. batrachorum*-type trichomonads, particularly *T. hominis*. (The writer is indebted to the following persons for furnishing source material from which certain of the trichomonads were isolated: C. M. Herman, U. S. Fish and Wildlife Service; E. E. Lund and D. K. McLoughlin, Animal Disease and Parasite Research Branch, ARS, USDA; Captain D. L. Price, MCS, USA; Miss B. Sonnenberg, Second Army Area Laboratory, Fort George G. Meade; and Miss L. V. Reardon, National Institute of Allergy and Infectious Diseases).—Louis S. Diamond, *Animal Disease and Parasite Research Branch, ARS, USDA, Beltsville, Maryland.*

SIMILAR INTERNAL MORPHOLOGY OF *TOXOPLASMA GONDII* AND *BESNOITIA JELLISONI* STAINED WITH SILVER PROTEIN

Toxoplasma gondii stained with hematoxylin or Giemsa shows little definite internal morphology besides a single nuclear structure and some irregularly distributed granules (Cross, 1949, J. Infect. Dis. **80**: 278–296). Gustafson *et al.* (1954, Am. J. Trop. Med. & Hyg. **3**: 1008–1021) found that thin sections of *Toxoplasma* examined with the electron microscope showed several additional internal structures previously undescribed. We have found, in the course of work which will be described in detail at a later date, that by using Honigberg and Davenport's modification of the Bodian silver protein method (1954, Stain Tech. **29**: 241–246), toxoplasmas show most of the structures seen by Gustafson *et al.* plus additional structures which were not seen in the electron microscope sections. Thus with this relatively simple technique there are a number of distinct morphological features by which *Toxoplasma* and toxoplasma-like organisms can be characterized.

Frenkel (1953, Atti 6th Cong. Intern. Microbiol. Rome **5**(XV): 426–434) described among other organisms resembling *Toxoplasma,* a new protozoan species, *Besnoitia jellisoni,* which was isolated from a field rodent. This organism is almost identical with *T. go 'ii* so far as

the shape and size of the proliferative form is concerned. It differs from *Toxoplasma* in the type of cysts it produces in chronic infections, in details of pathology in laboratory animals, and in its serology. Through the courtesy of Dr. Leon Jacobs of the National Institutes of Health, and Dr. J. K. Frenkel of the University of Kansas School of Medicine, we have obtained several infected mice and have been able to subject *B. jellisoni* to the same staining techniques we had used for *Toxoplasma*. The accompanying figures demonstrate the striking morphological similarity between the two organisms. Both species show distinctly the following: (1) A truncated cap-like cone at the anterior end; (2) one or more rod-like or fibril-like structures extending posteriorly from the cone; (3) a nucleus consisting of a larger, less dense portion, and a smaller, more compact structure which appears to be different from the usual nucleolus seen in other types of cells; (4) a dark staining posterior granule; and (5) a similar and, so far as we know, unique, method of reproduction. The last point refers to our finding that *Toxoplasma* reproduces, at least in the proliferative stage, by a process in which the parent cell develops within itself 2 daughter cells which are small duplicates of the parent. Eventually the daughter cells fill the anterior portion of the parent and break out, presumably



DESCRIPTION OF FIGURES

FIGURES 1 and 3 are of *T. gondii*. FIGURES 2 and 4 are of *B. jellisoni*. 1 and 2. "Resting" forms. 3 and 4. Reproducing forms.

destroying the original cell in the process. Evidence that this process takes place will be presented in the detailed paper to be published later, but 2 photographs (Figures 3 and 4) have been included here to show stages in the process both for *Toxoplasma* and *Besnoitia*.

The serological distinctiveness of the two organisms has been partially confirmed by us by the fact that fluorescein-labelled antibody to *Toxoplasma* did not stain *Besnoitia* at all under conditions in which *Toxoplasma* stained vigorously to yield brightly fluorescent organisms.

If these observations are corroborated there will remain little reason for doubting the close taxonomic relationship of *T. gondii* and *B. jellisoni*. In addition, Ludvík (1956, Zent. f. Bakt. I Abt. Orig. **166**: 60–65) has demonstrated by electron microscope studies that *Sarcocystis tenella* possesses certain structures similar to those shown by *Toxoplasma*. It seems likely that the application of the silver protein staining method to a variety of obscure, *Toxoplasma*-like organisms would greatly facilitate the establishment of a rational taxonomy for the group. To us, the most significant aspect of the brief studies reported here is that *Toxoplasma* no longer appears to be an isolated and essentially unclassified parasite of man and animals. Instead, it appears probable that it is just one species of a group of which the members may differ in some details but which are nevertheless closely related morphologically and biologically.—MORRIS GOLDMAN, R. K. CARVER, AND A. J. SULZER, *Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia.*

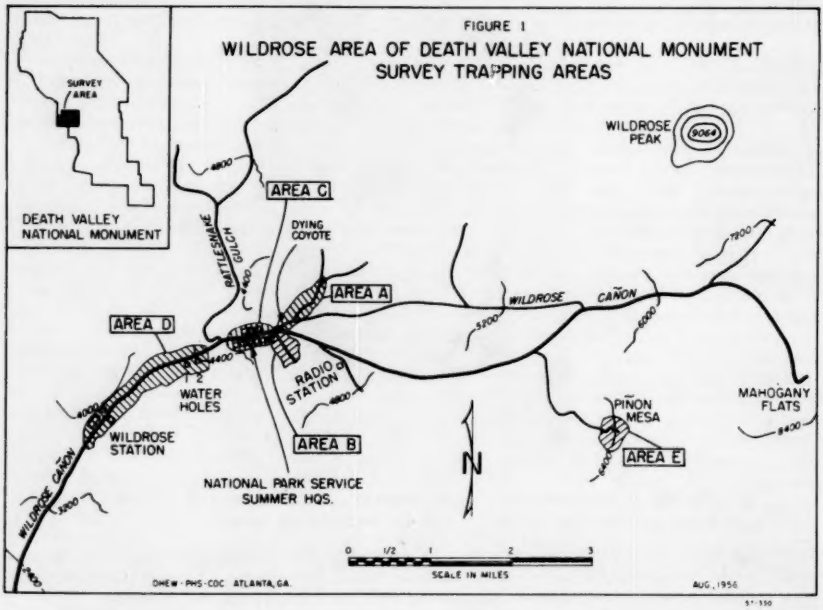
A RODENT–ECTOPARASITE–DISEASE SURVEY IN DEATH VALLEY NATIONAL MONUMENT, CALIFORNIA

At the request of the National Park Service, a survey was conducted in the Wildrose Canyon area of Death Valley National Monument from July 15 to July 19, 1956. The basis for the work was a report of a "die off" of wild animals, a circumstance noted by the authorities with some alarm because of a fatal human case of plague, from sylvatic sources, in Ventura County, California, during June, 1956.

Interviews with Death Valley officials and professional personnel, and data on animal sightings by rangers and tourists indicated the following: 1—a dying coyote was found and another sick-appearing animal of this species was seen; 2—there was no evidence that any other species of animal had been found dead, dying, or sick; 3—greater numbers of rabbits and hares were observed during 1955 than during a corresponding period in 1956; 4—no actual decline in numbers of wild rodents or other animals was noted; 5—there was an exceptionally severe drought in the Wildrose area during the summer of 1956.

Trapping with Museum Special type snap traps and the usual rat snap-trap was done in representative places in the Wildrose Canyon area as shown in Figure 1. Approximately equal

FIGURE 1



trapping efforts were made in all sites except in area E where it was about 2/3 that in other places. Each trapped animal was placed into an individual sack, brought to the laboratory, and subjected to cyanide gas. Its ectoparasites were removed, and the animal was then dissected and examined for lesions and portions of spleen and/or liver were removed and placed in sealed vials. In some cases, thick and thin blood smears were made from heart blood at the time of autopsy. Ectoparasites were placed in sealed vials with 2% saline and the tissues were placed in containers without any preservative. All material was stored in a refrigerator and transported to San Francisco in a portable icebox.

On July 23–24, tissue and ectoparasite species pools were inoculated separately into laboratory mice. The mice were held for about 2 weeks and were killed and examined for lesions and for the presence of pathogenic organisms in tissues. The blood films were fixed with absolute methyl alcohol and stained by the Giemsa method. At least 50 fields of the thick and 200 fields of the thin smears were examined under oil.

The following animals were either trapped, shot, or seen during the survey: Wild burro, *Equus assinus;* Coyote, *Canis latrans;* Antelope ground squirrel, *Citellus lecurus;* Panamint pocket mouse, *Perognathus longimembris panamintinus;* Western desert mouse, *Peromyscus*

eremicus eremicus; Wood rat, *Neotoma lepida;* Desert kangaroo rat, *Dipodomys deserti;* Pale dwarf pocket rat, *Microdipidops pallidus;* Arizona cottontail, *Sylvilagus auduboni arizonae;* Colorado desert jackrabbit, *Lepus californicus deserticola;* and Little pallid bat, *Myotis californicus pallidus.*

A total of 133 rodents was trapped in the 5 trapping areas. The number of individuals per species and the percentage of the total animals taken were as follows: *Perognathus longimembris,* 47 (35.3); *Peromyscus eremicus,* 33 (24.8); *Neotoma lepida,* 25 (18.8); *Citellus leucurus,* 15 (11.2); *Microdipidops pallidus,* 9 (6.7); *Dipodomys deserti,* 4 (3.2). In addition, 1 rabbit, *S. auduboni,* was shot in area C, and 1 each of *L. californicus* was shot in areas C and D. A well-fed and healthy-appearing coyote was seen in area A and in area E.

The majority of animals were taken in areas C and D where water was most abundant. Wood rats were prevalent under houses, as were kissing bugs, *Triatoma* sp., which commonly parasitize these rodents. The only animals harboring fleas were *Citellus leucurus,* with an average of 0.66 fleas composed of *Hoplopsyllus anomalus* and *Thrassis gladiolis,* and *Neotoma lepida* with an average of 0.24 fleas composed of *Orchopeas sexdentatus nevadensis.* All the fleas were taken on animals from areas with water supplies (*i.e.* C, D, E). Thus the drought appeared to be a limiting factor in flea production during the period of the survey.

The inoculation of rodent tissues and fleas did not reveal the presence of any disease. All the animals trapped or shot appeared in good condition and no evidence of parasites or other organisms was seen in 73 blood films examined. In general, the evidence did not suggest an epizootic die off, but it did indicate a situation in which animals and ectoparasites were engaged in a struggle for existence conditioned by the available water supply.—Leo Kartman and Alva R. Kinney, *U. S. Department of Health, Education, and Welfare, Public Health Service, Bureau of State Services, Communicable Disease Center, San Francisco Field Station, San Francisco 18, California.*

FURTHER OBSERVATIONS ON THE LIFE-CYCLE OF *GYNAECOTYLA ADUNCA* (LINTON, 1905)

There has been considerable confusion concerning the life history and systematic position of *Gynaecotyla adunca* (Linton, 1905) (Bull. Bur. Fish. **24:** 323–428). This confusion was compounded when Hunter and Vernberg (1953. Trans. Am. Micro. Soc. **72:** 163–170) erroneously reported a tailless xiphidiocercaria to be the larval stage of *G. adunca.* Subsequent work has shown that *Cercaria nassicola* Cable and Hunninen, 1940, an Ubiquita-type cercaria, penetrates the fiddler crab, *Uca pugilator,* and develops into the metacercaria of *G. adunca,* primarily in the green glands of this host. The cercariae were first observed being shed by species of *Nassarius vibex,* although later *N. obsoleta* also were found to be infected. This same cercaria was reported by Rankin (1940, Biol. Bull. **79:** 439–451) to be the larval stage of *G. nassicola* (Rankin, 1939). In this case, the cercaria penetrated and developed in *Talorchestia megalophthalmia.*

It has been impossible to secure a sufficient number of uninfected fiddler crabs; crabs measuring but a few mm in width often have 1 or 2 cysts or developing metacercariae. Larger forms are nearly 100% infected, usually quite heavily. In previous experiments, crabs were isolated in the laboratory for a minimum of 2 weeks; it was thought that this time interval would insure most of the cercariae reaching the encystment stage. This proved to be a grave error. Indeed, when crabs were examined which had been in the laboratory for over 4 months, unencysted forms were still present. None of these, however, could be confused with the earliest developmental stages; the forms found included those previously described as 48-, 72-, 96-hour stages and many which were in the process of early cyst-formation. Therefore, it was possible only to use these crabs which had been isolated for several months to obtain at least the very early stages in the development of the metacercariae. Cercariae were recovered from the green glands of such crabs within 12 hours after a snail shedding *C. nassicola* had been placed with the crabs; these larvae were identical with the cercariae in every respect except for the loss of the tail.

Developing metacercariae were recovered from the green glands of crabs exposed to cercariae 9 days previously, in which the stylet was still visible, and the body measured 0.175 mm long (the cercariae average 0.11 mm). Developing metacercariae in the same stage of differentiation and with the same body measurements were recovered after exposure to the cercariae 3 weeks previously. This would suggest that the route through the body leading to the green glands sometimes may be a devious one, and that the cercariae may circulate throughout the body of the crab for some time before entering the green glands. No viable developmental stages have been recovered as yet from any other part of the body than the green glands, although

several non-viable developmental stages were recovered from the muscle tissues. Another explanation for the presence of relatively undifferentiated larvae so long after exposure to the cercariae could be that the infection in the green glands is already so heavy that a new infection encounters an immune reaction as well as competition for nourishment and space. At any rate, it is at present impossible to set time limits on the development of the metacercariae. As stated above, unencysted metacercariae have been recovered from the green glands of crabs which had been isolated in the laboratory for a period of at least 4 months. The relative changes in size and morphology have been described in our previous paper (1953), but the time sequence stated there is certainly to be challenged.

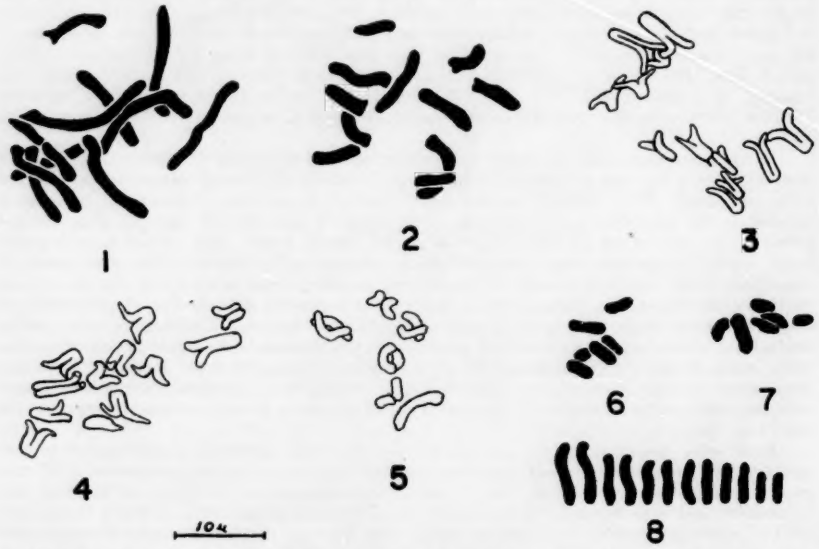
As was stated in our earlier paper, we believe *G. nassicola* is a synonym of *G. adunca*. Rankin (1939) distinguished *G. nassicola* from *G. adunca* primarily on the lobulation of the intestine and on the basis of body size, which fell within the range reported for *G. adunca* by Hunter (1952, J. Parasit. **38:** 308–314). Cysts and developmental stages have been recovered from specimens of *Talorchestia megalopthalmia* in the Beaufort area, and we are unable to differentiate them from *G. adunca*.—WANDA SANBORN HUNTER AND WINONA B. VERNBERG, *Duke University, Durham, North Carolina.*

THE CHROMOSOMES OF *HYMENOLEPIS DIMINUTA*

The chromosomes of this cestode were first examined by Jones (1945, J. Parasitol. **31:** 213–235). He reported that the chromosome number was probably 12, but results were not conclusive because of fixation difficulties. Until now, no confirmation of this number has been published.

The chromosomes of the first few cleavage divisions were studied in an attempt to determine the normal number and morphology. Meiosis was not thoroughly investigated at this time since the chromosomes are very small. Excellent fixation and staining were obtained when the worm was removed from the intestine of a freshly killed rat, washed in 0.85% saline, and immediately transferred to Carnoy's fixative (6:3:1—absolute alcohol, chloroform, acetic acid). After a 12-hour period of fixation, it was placed in 95% alcohol for 24 hours followed by a softening exposure of 1–2 hours in 45% acetic acid. Aceto-orcein squashes were made from selected regions containing developing embryos.

The chromosomes of 50 cells were drawn with the aid of a camera lucida at a magnification of 1800×. The stages ranged from late prophase to early anaphase. From these it was ascer-



DESCRIPTION OF FIGURES: 1–4, Mitosis in embryos; 1, Late prophase; 2, Metaphase; 3, 4, Early anaphase; 5, Diakinesis in an oocyte; 6, 7, Spermatocyte diakinesis; 8, Idiogram based on measurements of chromosomes like those of Fig. 2.

tained that the normal number was 12, thus confirming the report of Jones. Observation of chromosome movement during anaphase indicated that all were acrocentric. The average sizes of the chromosomes of 17 cells taken to be first cleavage, measured in microns for each pair are as follows: 7, 5.5, 5, 5, 4.5, 3. The chromosomes of later divisions appeared to decrease in size with each succeeding division. Occasionally a cell was observed in which there appeared to be one more or one less than the normal number.

This study was supported in part by the U. S. Atomic Energy Commission Contract No. AT (40-1) 1749.—RAYMOND L. KISNER, *Department of Zoology and Entomology, University of Tennessee, Knoxville.*

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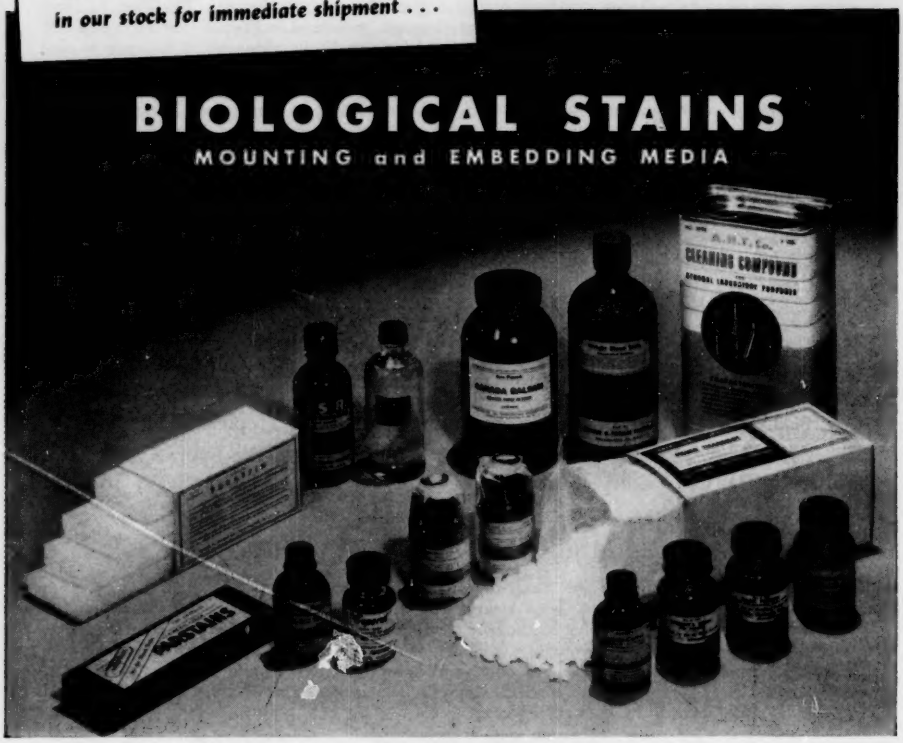

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